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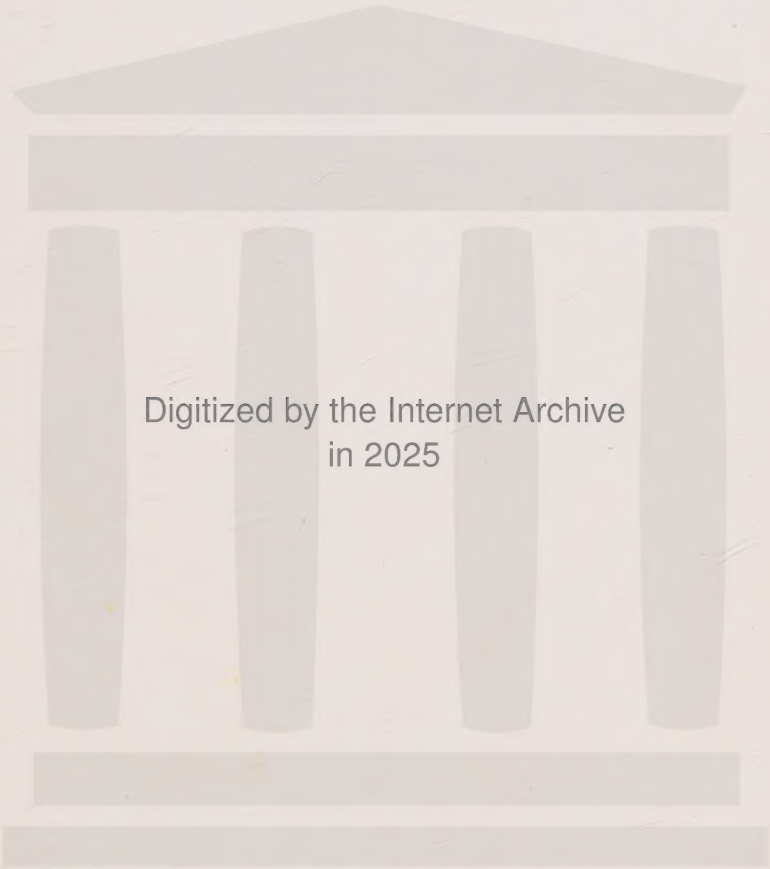
THE EARLY EMBRYOLOGY OF THE CHICK

PATTEN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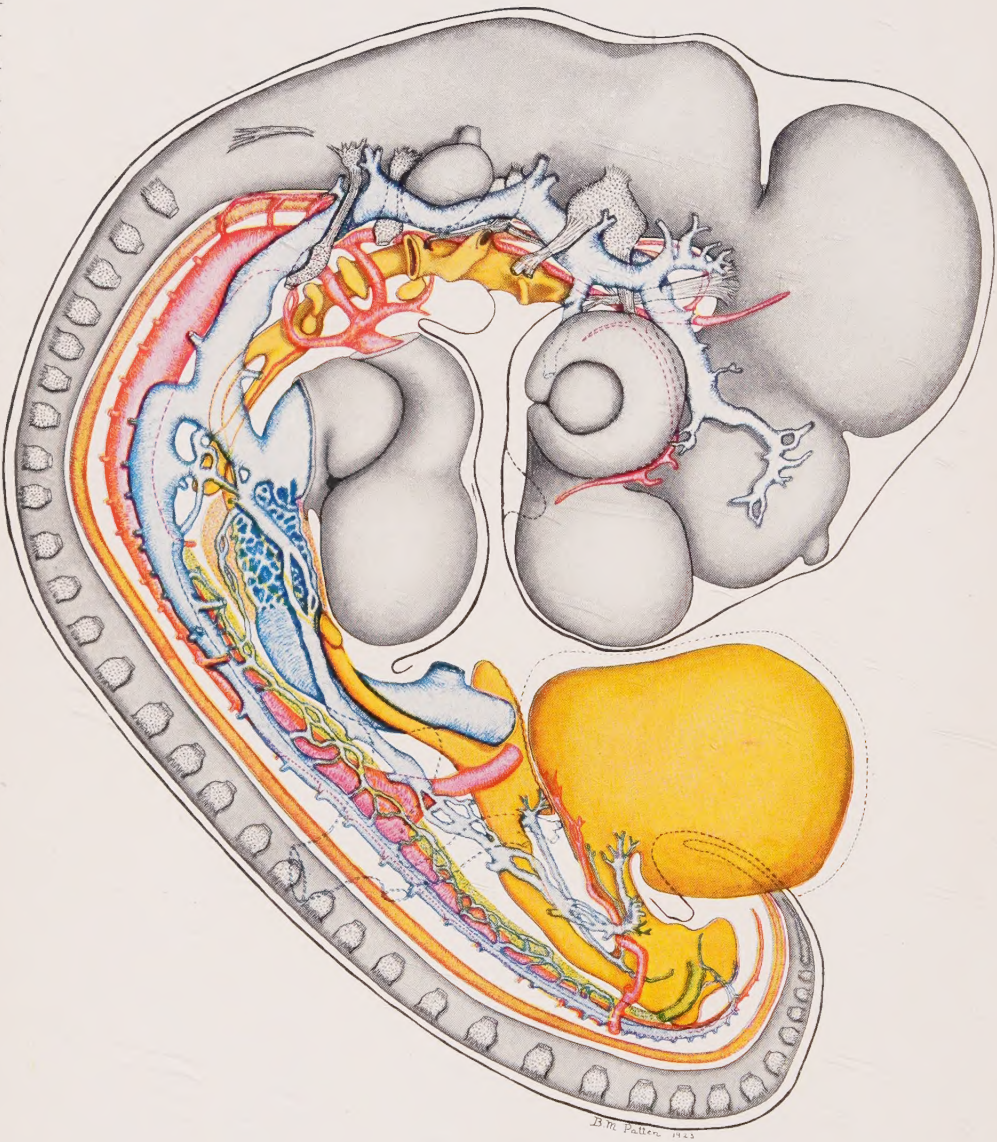

Chick Embryo of Four Days, Reconstruction x 51.

FRONTISPIECE

Drawn from a wax-plate reconstruction—original × 51, reproduced × 23.
For key to structures represented see Figures 70 and 71

THE EARLY EMBRYOLOGY

OF

THE CHICK

BY

BRADLEY M. PATTEN

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THIRD EDITION

WITH EIGHTY-SEVEN ILLUSTRATIONS CONTAINING TWO HUNDRED EIGHTY FIGURES

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PREFACE TO THIRD EDITION

The fact that chick embryos are so generally used as laboratory material for courses in vertebrate embryology seems to warrant the treatment of their development in a book designed primarily for the beginning student. To one commencing the study of embryology the very abundance of the information available is confusing and discouraging. He is unable to cull the essentials and fit them together in their proper relationships and is likely to become hopelessly lost in a maze of details. This book has been written in an effort to set forth for him in brief and simple form the basic facts of development. It does not purport to be a reference work. Details and controverted points have been avoided for the sake of clarity in outlining fundamental processes.

The story of development as illustrated by the chick, has been taken as the scheme of presentation. This departure from the conventional comparative method of approach does not imply an undervaluation of the comparative point of view. On the contrary, I am convinced that it is the goal to be sought by anyone who would consider himself trained in embryology; and, wherever it seemed possible without breaking the thread of the story of development, processes have been interpreted from this broader outlook. But as a basic scheme of presentation, following the development of several forms simultaneously seems to me an irrational method for the beginner. A student new to the subject is both uninterested and confused when confronted at the outset by a mass of comparative data. Comparisons, when the facts compared are unfamiliar and their individual significance is but vaguely understood, mean little or nothing. They make but a superficial scratch on the brain, which, in a healthy young individual, heals with amazing promptness and leaves no scar. What a beginner needs is not a vast array of facts to be memorized but the thread of a coherent story to hold together the new facts he acquires, and

a comprehension of their significance which will make them lastingly his own. The story of the development of a single form, told without digression, has an inherent quality of sustained interest which is of inestimable value in creating an understanding of embryological processes. Building on such a foundation, each new excursion into the broader field of comparative embryology becomes more stimulating because its findings are progressively more significant.

Because of my conviction that so-called elementary texts too frequently overreach their avowed scope, the ground covered by this book has been rigidly restricted. This curtailment will be most evident in the treatment which has been accorded to the very early and the relatively late phases of development. With the rapid advances made during recent years in cytology and in genetics, much material has been accumulated which has an interesting bearing on gametogenesis and maturation as processes leading toward the initiation of development. In this book no attempt has been made to go exhaustively into these phases of the subject. At the present time the extent to which pre-embryological processes are dealt with varies greatly in different courses and their treatment can best be worked out by the individual instructor according to his interests. Moreover, I have the feeling that the continued growth of our knowledge will soon lead to the establishment of special courses dealing with this important field that overlaps the boundaries of cytology, genetics and embryology.

The detailed account of development has been carried only through the first four days of incubation, the later developmental processes being dismissed with mere statements as to the adult structures that are derived from the various organs of the embryo. The reasons for thus devoting the major part of the book to the early phases of development would seem quite obvious. In this period the body of the embryo is laid down and the organ systems are well established. To one at all familiar with the adult structure of vertebrates it is relatively easy to understand the later changes in the position and proportions of organs if he has seen how they are first formed. Furthermore, courses in elementary embryology rarely continue work on the chick beyond the early stages of its development, and more extensive courses, in which a knowledge of mammalian

embryology is the objective, ordinarily pass from the study of three or four day chicks to work on mammalian embryos.

While the text has been kept brief, illustrations have been freely used in the belief that they convey ideas more readily than can be done in writing. Much time and care has been used in an effort to make each figure convey its message with clearness and accuracy. Most of the drawings were made directly from preparations in the laboratory of Histology and Embryology of Western Reserve University School of Medicine. However, figures from other authors, particularly Lillie and Duval, have been used extensively for comparisons and for schemes of presentation. Several figures have been reproduced directly or with only slight modifications. These are designated in the figure legends.

I wish to acknowledge the assistance I received in the preparation of material by Mrs. Mary V. Bayes, and in the drawing of the figures by Mrs. Bayes and Dr. Louis J. Karnosh. I am also indebted to my father, Prof. Wm. Patten of Dartmouth College, for criticism of the figures, and to Dr. F. C. Waite of the School of Medicine, Western Reserve University, for his helpful interest and coöperation in all phases of the preparation of the book and especially for his reading of the manuscript.

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CHAPTER I

INTRODUCTION

Embryology.—Every one of the higher animals starts life as a single cell,—the fertilized ovum. This fertilized ovum, as its technical name zygote implies, has a dual origin. It is formed by the fusion of a germ cell from the male parent with one from the female parent. The union of two such sex cells to form a zygote constitutes the process of fertilization and initiates the life of a new individual. Embryology is the study of the growth and differentiation undergone by an organism in the course of its development from a single fertilized egg cell into a highly complex and independent living being like its parents.

As a study embryology offers more than the mere acquisition of an encyclopedic array of facts. It gives one an understanding of some of the ways of life. We are all egoists, to a certain extent at least, and anything that touches the matter of our own whence and whither is of absorbing interest. The processes by which a fish, or an alligator, or a chick grows from a single fertilized egg-cell to its fully elaborated adult structure are fundamentally the same as those involved in our own development (Fig. 1). And these growth processes hold for us something definite and tangible in answer to that ever recurring question, "Whence do we come and how?"

Embryology is also an important source of evidence as to the path followed by Evolution. It tells us in one short, uninterrupted story how each individual grows into an adult. We can see this process going on under our very eyes. And we know that the story of individual development sketches for us in outline the evolutionary changes of our forbears. For the law of biogenesis or recapitulation is that ***every living thing in its individual development, passes through a series of constructive stages like those in the evolutionary development of the race to which it belongs.*** "This means that there is but one main way to upbuild a given kind of organism, and that every

individual must do it in essentially the same way his ancestors did. But to-day the individual can do this much more quickly and

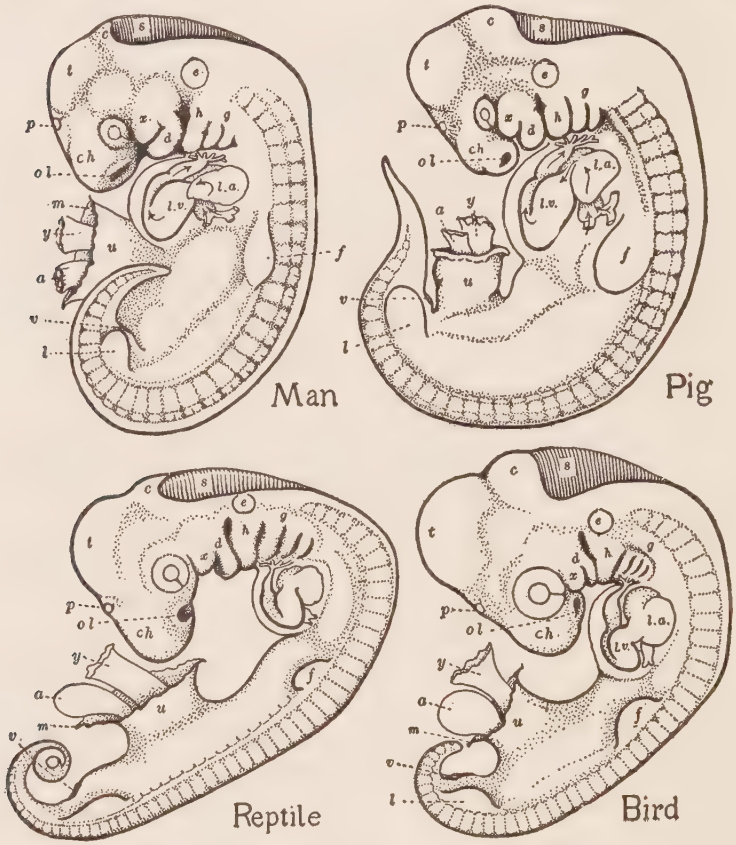


FIG. 1.—Embryos of man, pig, reptile, and bird at corresponding developmental stages. The striking resemblance of the embryos to one another is indicative of the fundamental similarity of the processes involved in their development.

ABBREVIATIONS

a, allantois
c, cerebellar region of brain
ch, cerebral hemispheres
d, mandible
e, ear
f, fore-limb
g, pharyngeal region
h, hyoid arch
l, hind limb
l.a., left atrium
l.v., left ventricle
m, cut margin of amnion
ol, olfactory pit
p, pineal body
s, thin roof of hind-brain
t, optic and auditory brain center
u, umbilical cord
v, urogenital opening
y, yolk-stalk

economically than its ancestors, because it does not have to find out by long and costly experimenting just how to do it.

Essentially the right ways and means of doing it 'automatically' are provided for it by its antecedents. At every step in the process it is using something which Nature, out of all the efforts of the past, has provided for it. These ways and means constitute the Heritages of individual life, and range all the way from ancestral germinal materials, stored up foods, and other parental provisions, to the environmental, physical and social provisions in which the growing organism is placed."[1]

More tangible perhaps than the outlook embryology gives on the life of today and on its evolutionary history in the past, is its direct help in the study of anatomy. Not until the student becomes enmeshed in a maze of structural details does he realize his imperative need of a knowledge of how and why adult conditions became as they are. For only this knowledge will lead him beyond blind memorizing to comprehension. And when he delves still deeper into anatomy he begins to encounter puzzling variations from the normal body architecture. Sometimes these are merely minor anomalies which do not materially affect the functional fitness of the individual; sometimes they are extensive malformations which render continued life precarious or even altogether impossible. Our present understanding of such conditions, and what future hope there may be of reducing the frequency with which they occur can come only through embryology.

The only method of attaining a comprehensive understanding of embryological processes is through the study and comparison of development in various animals. Many phases of the development of any specific organism can be interpreted only through a knowledge of corresponding processes in other organisms. The beginning student, however, can most readily and with least risk of confusion acquire his knowledge of embryology through intensive study of one form at a time. Building on the familiarity with fundamental processes of development thus acquired, he may later broaden his horizon by the comparative study of a variety of forms.

The Chick as Laboratory Material.—The chick is one of the most satisfactory animals on which student laboratory work in embryology may be based. Chick embryos in a proper state of preservation and of the stages desired can readily be

[1] From William Patten, "Evolution."

secured and prepared for study. Used as the only laboratory material in a brief course they afford a basis for understanding the early differentiation of the organ systems and the fundamental processes of body formation common to all groups of vertebrates. In more extended courses where several forms are taken up, the chick serves at once as a type for the development characteristic of the large-yolked eggs of birds and reptiles, and as an intermediate form bridging the gap between the simpler processes of development in fishes and amphibia on the one hand and the more complex processes in mammals on the other. In medical school courses where a knowledge of human embryology is the end in view the chick not only makes a good stepping stone to the understanding of mammalian embryology, but also provides material for the study of early developmental processes not readily demonstrable in mammals.

Plan and Scope of This Book.—This book on the development of the chick has been written for those who are beginning the study of embryology and has accordingly been kept as brief and uncomplicated as possible. Nevertheless it is assumed that the beginner in embryology will not be without a certain back-ground of zoölogical knowledge and training. He may reasonably be expected to be familiar with the fundamental facts of evolution and heredity, the structure of cells and their methods of division, the nature of the various types of tissues, and the more general phases of the morphology of vertebrates. It, therefore, seems unnecessary to include here any preliminary discussion of these phenomena. References for collateral reading on this and other phases of the subject are given in the appendix.

Methods of Study.—Like other sciences embryology demands first of all accurate observation. It differs considerably, however, from such a science as adult anatomy where the objects studied are relatively constant and their component parts are not subject to rapid changes in their inter-relations. During development, structural conditions within the embryo are constantly changing. Each phase of development presents a new complex of conditions and new problems.

Solution of the problems presented in any given stage of development depends upon a knowledge of the stages which precede it. To comprehend the embryology of an organism

one must, therefore, start at the beginning of its development and follow in their natural order the changes which occur. At the outset of his work the student must realize that proper sequence of study is essential and may not be disregarded. A knowledge of structural conditions in earlier stages than that at the moment under consideration, and an appreciation of the trend of the developmental processes by which conditions at one stage become transmuted into different conditions in the next, are direct and necessary factors in acquiring a real comprehension of the subject. Without them the story of embryology becomes incoherent, a mere jumble of confused impressions.

A knowledge of the phenomena of development is ordinarily acquired by studying a series of embryos at various stages of advancement. Each stage should be studied not so much for itself, as for the evidence it affords of the progress of development. In the study of embryology it does not suffice to acquire merely a series of "still pictures" of various structures, however accurate these pictures may be. The study demands a constant application of correlative reasoning and an appreciation of the mechanical factors involved in the relations of various structures within the embryo to each other, and in the relation of the embryo as a whole to its environment. In order really to comprehend the embryological significance of a structure one must know not only its relations within the embryo being studied at the time, but also the manner in which it has been derived and the nature of the changes by which it is progressing toward adult conditions. To get absolutely the whole story it is obvious that one would have to study a series of embryos with infinitely small intervals between them. Nevertheless the fundamental steps in the process may be grasped from a much less extensive series. The fewer the stages studied, however, the more careful must one be to keep in mind the continuity of the processes and to think out the changes by which one stage leads to the next.

The outstanding idea to be kept in mind by the student beginning the study of embryology is that the development of an individual is a process and that this process is continuous. The conditions he sees in embryos of various stages are of importance chiefly because they serve as evidence of events in the process

of development at various intervals in its continuity, as historical events are evidences of the progress of a nation. Just as historical events are led up to by preparatory occurrences and followed by results which in turn affect later events, so in embryology events in development are presaged by preliminary changes and when consummated affect in turn later steps in the process.

In certain respects the laboratory study of embryological material involves methods of work for which courses in general

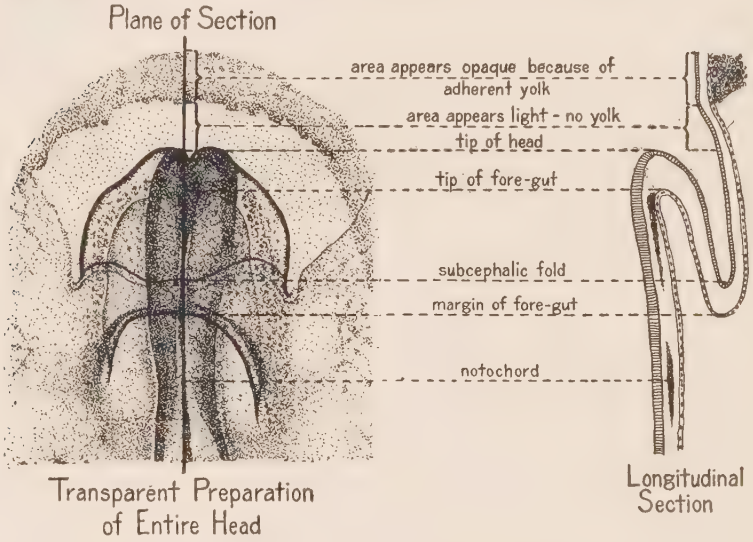


FIG. 2.—Relation of a longitudinal section of the embryonic head to the picture presented by a head of the same age mounted entire as a semi-transparent preparation.

zoölogy do not entirely prepare the student. Some general suggestions as to methods of procedure are, therefore, not out of place.

In dissecting gross material it is not unduly difficult to appreciate the complete relationships of a structure. The nature of embryological material, however, introduces new problems. Embryos of the age when the establishment of the various organ systems and processes of body formation are being initiated are too small to admit of successful dissection, but not sufficiently small to permit of the satisfactory microscopical study of an entire embryo, except for its more general organization. To study embryos of this stage with any degree

of thoroughness they must be cut into sections which are sufficiently thin to allow effective use of the microscope to ascertain cellular organization and detailed structural relationships. In preparing such material the entire embryo is cut into

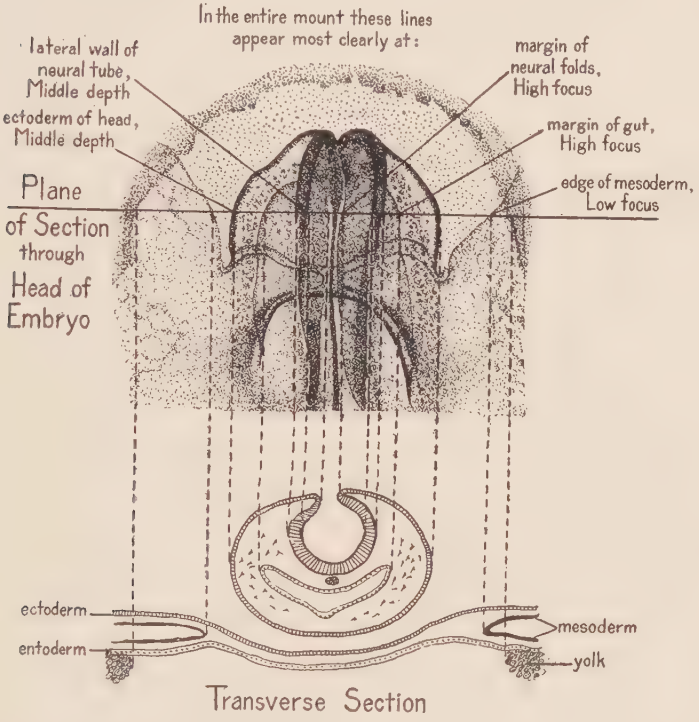

In the entire mount these lines appear most clearly at:
lateral wall of neural tube, Middle depth
ectoderm of head, Middle depth
Plane of Section through Head of Embryo
margin of neural folds, High focus
margin of gut, High focus
edge of mesoderm, Low focus
ectoderm
entoderm
mesoderm
yolk
Transverse Section

FIG. 3.—Relation of a transverse section of the embryonic head to the picture presented by an entire head of the same age viewed as a semi-transparent preparation. The two drawings may be brought into closer relation by looking at them with the top of the page tilted downward.

This and the preceding figure show the aid that sections can be in interpreting entire embryos, and at the same time the way in which the greater perspective lent by study of entire embryos increases the significance of sectional pictures. Note especially that neither the transverse nor the longitudinal section alone adequately interprets all the features of the entire mount. An embryo is a three-dimensional object and must be studied with that fact in mind. These figures show also how largely the appearance of lines or bands seen in studying transparent mounts of entire embryos is due to looking edgewise through a folded layer.

sections which are mounted on slides in the order in which they were cut. A sectional view of any region of the embryo is then available for study.

While sections readily yield accurate information about local regions it is extremely difficult to construct a mental picture

of any whole organism from a study of serial sections alone. For this reason it is necessary to work first on entire embryos which have been prepared by staining and clearing so they may be studied as transparent objects. From such preparations it is possible to map out the configuration of the body, and the location and extent of the more conspicuous internal organs. In this work the fact that embryos have three dimensions must be kept constantly in mind and, by careful focusing, the depth at which a structure lies must be determined as well as its apparent position in surface view. While conventionally entire chick embryos are represented in dorsal view, much additional information may be gained by following a study of the dorsal, with a study of the ventral aspect. Unless the preliminary study of entire embryos is carefully and thoroughly carried out the study of sections will yield only confusion.

In studying a section of an embryo it is necessary first of all to determine its location. The plane of the section under consideration, and the region of the embryo through which it passes should be ascertained by comparing it with an entire embryo of the same age as that from which the section was cut. Only when the exact location of a section is known can the structures appearing in it be correlated with the organization of the embryo as a whole. Probably nothing in the study of embryology causes students more difficulties than neglect to locate sections accurately, with the consequent failure to appreciate the relationships of the structures seen in them. Too great emphasis cannot be laid on the vital importance of fitting the structures shown by sections properly into the general scheme of organization as it appears in whole-mounts (Figs. 2 and 3). It must by no means be inferred that the possibilities of the whole-mounts have been exhausted by the preliminary study accorded them before taking up the work on sections. Further and more careful study of entire embryos should constantly accompany the study of serial sections. Many details which in the initial observation of the whole-mount were inconspicuous or abstruse will become significant in the light of the more exact information yielded by the sections.

In embryology it is necessary to designate the location of structures and the direction of growth processes by terms which are referable to the body of the embryo regardless of the

position it occupies. Our ordinary terms of location which are referred to the direction of the action of gravity, such as above, over, under etc. are not sufficiently accurate because of the fact that the embryo itself may lie in a great variety of positions.[1] The correct adjectives of position, are dorsal, pertaining to the back; ventral pertaining to the belly; cephalic to the head; caudal to the tail; mesial to the middle part; and lateral to the side of the embryonic body. Adverbs of fixed position are made in the usual way by adding -ly to the root of the adjective. In addition to adverbs of position, corresponding adverbs of motion or direction are formed by adding the suffix -ad to the root of the adjective, as dorsad meaning toward the back, cephalad meaning toward the head, etc. These adverbs should be applied only to the progress of processes, or to the extension of structures toward the part indicated by their root. Thus, for example, we should say that the developing eye of an embryo was located in the later*al* wall of the fore-brain or that the fore-brain was in the cephal*ic* part of the embryo; but if we wished to express the idea that as the eye increased in size it moved farther to the side we should say it grew later*ad*. Cultivation of the use of correct and definite terms of position and direction in dealing with embryological processes will greatly aid accurate thinking and clear understanding.

[1] In gross human anatomy there still persist many terms that are referred to gravity. Such terms, because of the erect posture of man, are not applicable to comparative anatomy or to embryology. The most confusing of these are anterior and posterior as used to mean, respectively, pertaining to the belly and to the back. In comparative anatomy and in embryology, anterior has reference to the head region and posterior to the tail region. Because of this possible source of confusion, the terms anterior and posterior should be replaced by their more precise synonyms, cephalic and caudal.

CHAPTER II

THE GAMETES AND FERTILIZATION

CONTINUITY OF GERM PLASM; HISTORY OF SEX CELLS WITHIN THE PARENT BODY; SPERMATOGENESIS; OÖGENESIS; SIGNIFICANCE OF MATURATION; SEX DETERMINATION; FERTILIZATION; THE FORMATION OF THE ACCESSORY COVERINGS OF THE OVUM; THE STRUCTURE OF THE EGG AT THE TIME OF LAYING; INCUBATION.

Continuity of Germ Plasm.—The reproductive cells which unite to initiate the development of a new individual are known as gametes. In the case of all the higher organisms the gametes are of two types produced by two sexually differentiated individuals, the small, actively motile gametes from the male being called spermatozoa or spermia, and the larger, food-laden gametes formed within the female being termed ova. The gametes and the cells which give rise to them are said to constitute the individual's germ plasm. The cells which take no direct part in the production of gametes are called somatic cells. In antithesis to the germ plasm, the somatic cells collectively are said to constitute the somatoplasm.

The germ plasm is of paramount interest, not only to the biologist but to all thinking persons, because while the somatic cells cease to exist with the death of the individual whose body they constitute, the germ plasm may live on indefinitely in succeeding generations. In the higher vertebrates where sexual reproduction is the rule, the germ plasm of a single individual cannot survive by itself. There must be successful union of a male with a female sex cell. These two conjugating gametes alone pass on the entire hereditary dowry of the species. It is not easy to realize fully all that is implied by this simple statement as to the continuity of the germ plasm. It may make it more vivid if we apply it specifically to ourselves and say that the future of the human race depends on the germ plasm held in trust within the bodies of the individuals

now living. Fortunately, very early in the life of an individual, the germ plasm is segregated in the gonads and is not subject to most of the vicissitudes and the diseases from which the somatic cells suffer. But the germ plasm, even though it is not directly affected, may nevertheless suffer indirectly because of a poor environment forced upon it by an unhealthy body.

The quality of the germ plasm of any one individual, while of great importance, is only half the story. Of equal significance is the nature of the combination of germ plasm which occurs in each generation when the male and female gametes fuse. As surely as either gamete brings into the new combination defective germ plasm, so surely will both the body and the germ plasm of the new individual suffer therefrom.

History of Sex Cells within the Parent Body.—It is, therefore, of fundamental interest to go back of the production of gametes in the sexually mature individual so we may see all the steps in the preservation and transmission of the racial heritage. Obviously the germ plasm of any individual must have come to it from its parents by way of the ovum and the spermium. But one wants to know how and where the germ plasm was cared for by the parents; when in their life history it first became possible to recognize it as distinct from the somatoplasm; when and how it became segregated in the gonads; and what it was doing during the long period before sexual maturity.

There is still much of the very early history of the germ plasm which is but imperfectly known. Yet the cells which are destined to give rise to the gametes are definitely recognizable at a surprisingly early stage in development. Even before it is possible to tell whether an embryo is destined to become a male or a female, certain large cells, different from their neighbors, can be recognized as the primordial sex cells. In other words we know that they constitute the germ plasm as it exists in an embryo of that age.

The primordial sex cells are first easily identifiable when the gonads are just taking shape as definite organs. Until recently it was believed that, in the higher vertebrates, they could not be recognized as potential germ cells any earlier in their history. Recently much more detailed investigations have been made which seem to indicate that the sex cells can be

recognized even prior to their appearance in the newly formed gonad. According to these studies the primordial germ cells become recognizably differentiated in the wall of the yolk-sac, and migrate thence to become established in the growing gonads (Fig. 4). How much farther back toward the fertilized ovum the lineage of the primordial sex cells may in the future be traced it would be unwise to predict. Even now, in some of the invertebrates, it is believed that the single cell which gives rise to all the sex cells can be identified when the embryo is so young that it is no more than a minute ball of cells.

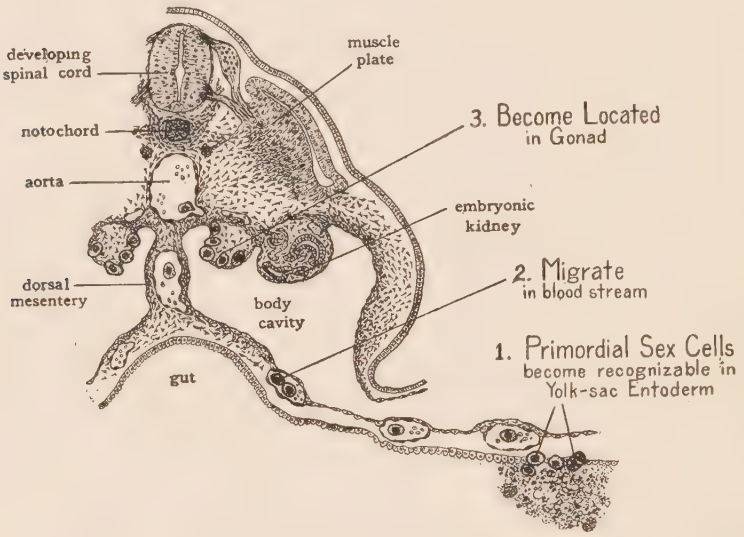


FIG. 4.—Schematic section through the mid-body region of a young embryo illustrating the manner in which the primordial germ cells are believed to originate in the yolk-sac entoderm and migrate thence to the developing gonad.

The upper part of the chart appearing as figure 5 summarizes graphically the conception of the early separation from the somatic cells, of certain cells which are destined to give rise to the gametes. As this process occurs before sexual differentiation we may take it as a common starting point for the germ-cell-lineage of either sex. The lower part of the chart outlines separately for each sex the later history of the germ cells. Sexual differentiation of the embryo begins to be apparent shortly after the primordial germ cells are established in the gonads. If the individual is to become a male the gonads differentiate into testes. During early embryonic life the

primordial sex cells become organized within the testis in the form of tortuous tubules called the seminiferous tubules. During the period of body growth which precedes sexual

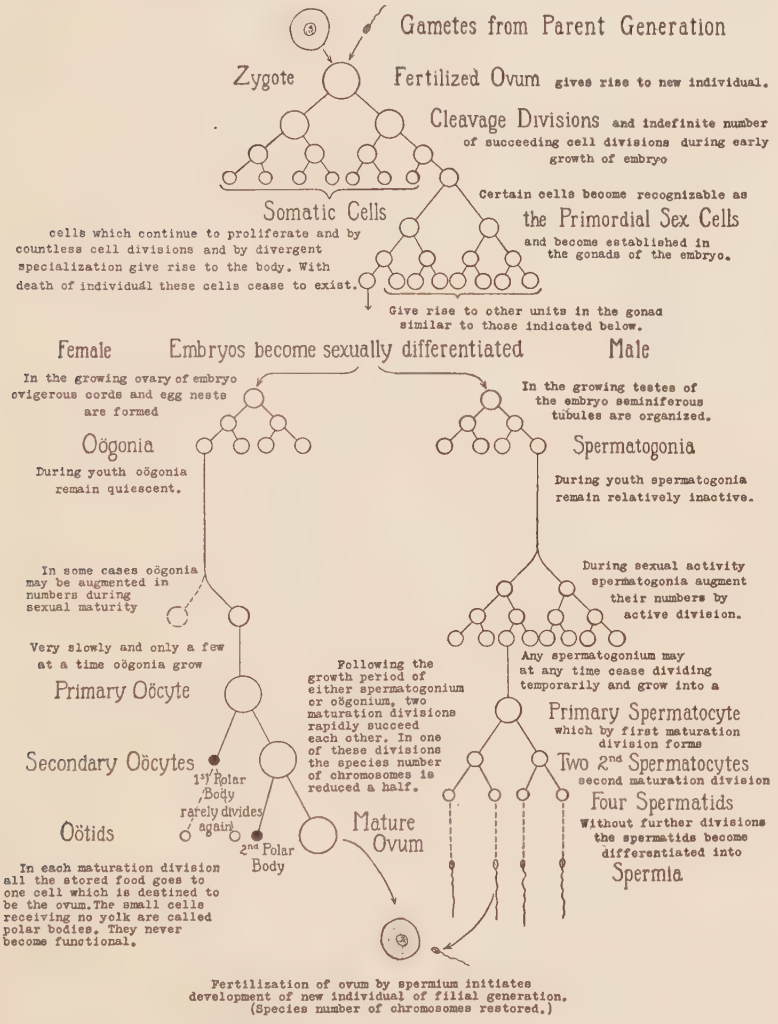

Gametes from Parent Generation
Zygote
Fertilized Ovum gives rise to new individual.
Cleavage Divisions and indefinite number of succeeding cell divisions during early growth of embryo
Somatic Cells
cells which continue to proliferate and by countless cell divisions and by divergent specialization give rise to the body. With death of individual these cells cease to exist.
Certain cells become recognizable as the Primordial Sex Cells and become established in the gonads of the embryo.
Give rise to other units in the gonad similar to those indicated below.
Female
Embryos become sexually differentiated
Male
In the growing ovary of embryo ovigerous cords and egg nests are formed
In the growing testes of the embryo seminiferous tubules are organized.
Oögonia
During youth oögonia remain quiescent.
Spermatogonia
During youth spermatogonia remain relatively inactive.
In some cases oögonia may be augmented in numbers during sexual maturity
During sexual activity spermatogonia augment their numbers by active division.
Very slowly and only a few at a time oögonia grow
Primary Oöcyte
Following the growth period of either spermatogonium or oögonium, two maturation divisions rapidly succeed each other. In one of these divisions the species number of chromosomes is reduced a half.
Any spermatogonium may at any time cease dividing temporarily and grow into a
Primary Spermatocyte
which by first maturation division forms
Two 2nd Spermatocytes
second maturation division
Four Spermatids
Without further divisions the spermatids become differentiated into
Spermia
Secondary Oöcytes
1st Polar Body
rarely divides again
Oötids
2nd Polar Body
Mature Ovum
In each maturation division all the stored food goes to one cell which is destined to be the ovum. The small cells receiving no yolk are called polar bodies. They never become functional.
Fertilization of ovum by spermium initiates development of new individual of filial generation. (Species number of chromosomes restored.)

FIG. 5.—Chart outlining, for one generation, the history of the gametes and the germ plasm from which they are derived.

maturity, the future gamete-producing cells or spermatogonia in these tubules remain quiescent. During the period of active sexual life the spermatogonia multiply and groups of them are constantly undergoing the final changes which precede their liberation as mature gametes (Fig. 5).

The changes undergone by the female sex cells are, in a general way, comparable to those passed through by the male cells. The large primordial cells of the germ plasm form cords and clusters in the embryonic ovary, homologous with the seminiferous tubules in the testis (Fig. 5). Not all the cells in these masses are destined to become functional gametes because many of them, speaking figuratively, forego their share of food and act as protective and food-purveying cells to the chosen few on which special care is lavished. All of these cells, however, are to be regarded as potential oögonia homologous with the spermatogonia of the male. The selection of certain oögonia for special nutrition is an early expression of the most characteristic difference between the male and female gametes. The male gametes contain no stored food material and are small and active. There is no special nutritional provision for any favored few. But such enormous numbers of them are produced that under normal conditions there is little chance that an elaborately prepared female gamete will go to waste because it is not found and fertilized by a spermium. The development of ova is on a quite different basis. Of all the potential gametes contained in the ovary, relatively few are destined to be brought to maturity. But these few are richly endowed with stored food materials. The energy expended by the male on quantity production of small active gametes ensuring fertilization, is thus, in the female, devoted to laying up a supply of food which provides the necessary materials for the growth of the embryo which is to follow fertilization.

Spermatogenesis.—The latter part of the history of the sex-cells which occurs just before their liberation as mature gametes demands closer scrutiny. In the male these terminal changes are spoken of as constituting the process of spermatogenesis. The cells of the germ line which came to be located in the seminiferous tubules of the growing testis we have already learned to know by their technical name of spermatogonia (Fig. 5). When a male animal becomes sexually mature these spermatogonia begin to produce gametes. They do not all become active at any one time, but periodically groups of spermatogonia begin to produce successive crops of mature spermia.

The process is much the same in all the higher vertebrates and it will be at once simpler and more profitable if we trace it in broad general lines rather than attempting to master its details in any one form. In sections of the testis, spermatogonia are found peripherally located in the walls of the seminiferous tubules. When a spermatogonium undergoes mitosis (Fig. 6, *A*, 1-3) either of the resulting cells may do one of two things. It may cease dividing for a time, and by growing to a size markedly larger than that of its parent, become differentiated as a primary spermatocyte (Fig. 6, *A*, 4). Or it may remain like its parent (Fig. 6, *A*, 3*a* and Spg.) and take the place of cells which crowd toward the lumen of the tubule (Fig. 6, *A*, 3*b*) to become differentiated into spermatocytes. Some of the spermatogonial cells always remain thus in the peripheral part of the tubule and furnish a constant source of new cells ready for conversion into spermatocytes.

Once a cell has passed through the growth phase which so differentiates it that it is called a primary spermatocyte its future history is definitely determined. As soon as its growth is completed it undergoes two mitotic divisions in rapid succession. The first of these divisions results in the formation of two smaller cells called secondary spermatocytes (Fig. 6, *B*, 7*a*, 7*b*). Each of these secondary spermatocytes, without any resting period which might allow the cells to grow to the size attained by their parents, promptly divides again. The four small cells thus produced from the primary spermatocyte by two successive cell divisions are known as spermatids (Fig. 6, *C*, 8*a*, *b*, *c*, *d*). The two divisions are known as the maturation (meiotic) divisions both because they are the last divisions that these cells undergo and because they accomplish certain important internal changes preparatory to the part these cells are to play in fertilization. The nature of these changes, especially as they affect the chromosomal content of the cells, we shall consider in more detail in connection with the similar changes taking place in the maturation of the female sex cells.

Although cell division ceases with the two maturation divisions the spermatids still have radical changes to undergo before they are capable of carrying out their function. During this metamorphosis of the spermatid the nuclear material becomes

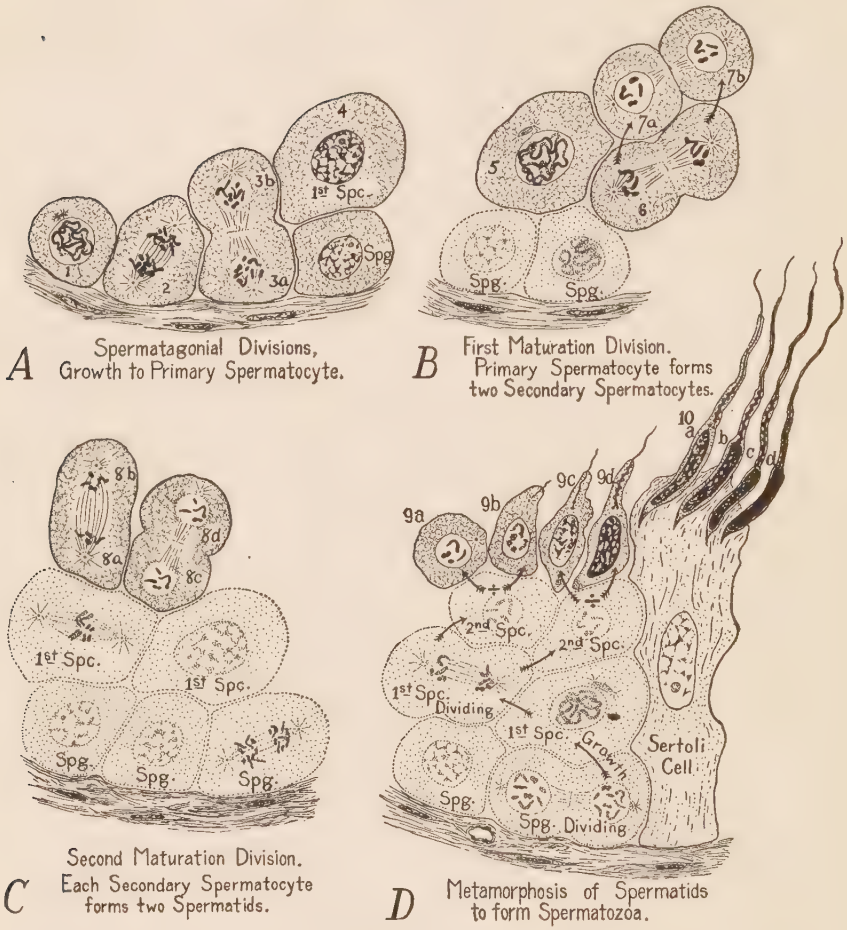


FIG. 6.—Semi-schematic diagrams to show the main steps in the progress of spermatogenesis. In the wall of a mature seminiferous tubule, cells in all stages of spermatogenesis occur in such close association that it is not always easy to grasp their relations. To obviate this difficulty these diagrams start with conditions in a young tubule just going into activity and follow the process a step at a time. The sequence of events is further indicated by consecutive numbering of the cells. In each part of the figure, cells undergoing the critical changes there emphasized are drawn in full detail, while cells not at the moment under consideration are "shadowed in" to make evident the accompanying changes in positional relations. For the sake of simplicity the species number of chromosomes is assumed to be eight.

ABBREVIATIONS

1st spc., primary spermatocyte
2nd spc., secondary spermatocyte
spg., spermatogonium.

very compact to form the bulk of the head of the spermium. The flagella-like tail is formed by the development of a contractile fibril which first makes its appearance in the cytoplasm near the centrosome and then rapidly grows in length till it projects far beyond the original confines of the cell. As this tail filament grows it remains enveloped in a thin film of cytoplasm which eventually forms a membrane covering all but the extreme tip of the tail (Fig. 6, *D*, 9*a-d*, 10). The modified centrosomal apparatus becomes located in the so-called "neck region" where the "tail" (flagellum) is joined to the "head" of the spermium. The cytoplasm of the spermatid is reduced greatly in bulk forming an almost invisibly thin envelope about the nuclear material of the head. At the extreme tip of the head a cap-like thickening of modified cytoplasm constitutes the acrosome. Thus a mature male gamete is a cell consisting essentially of a very compact nucleus provided with a flagellum which gives it the power of active locomotion in a fluid medium (Fig. 7).

FIG. 7.—Spermatozoön of the piegon. (*After Ballowitz.*)

Oögenesis.—Spermatogenesis could be dealt with in general terms because it is fundamentally the same in the entire vertebrate group. The processes involved in the formation of ova, while they also show a certain underlying similarity in all the great groups of vertebrates, are greatly modified by differences in the amount of food material stored as yolk in the egg cells. The amount of yolk characteristically present in the egg of any group of animals affects not only the manner in which the egg itself differentiates but also the developmental processes that follow fertilization. As we are going to devote our attention to the embryology of the chick which develops from an exceedingly large-yolked egg we must see how this yolk is accumulated during oögenesis and how its presence modifies the structure of the ovum, so that we may better understand the profound influence which a large yolk mass exerts on the early growth processes of the embryo.

The part of the hen's egg commonly known as the "yolk" is a single cell, the female sex-cell or ovum. Its enormous size as compared with other cells is due to the food material it

contains. This food material, or deutoplasm, destined to be used by the embryo in its growth, is gradually accumulated within the cytoplasm of the ovum before it is liberated from the ovary. Under the microscope it has the appearance of a viscid fluid in which are suspended granules and globules of various sizes. As the deutoplasm increases in amount the nucleus and the cytoplasm are forced toward the surface so that eventually the deutoplasm comes to occupy nearly the entire cell.

The significance of the chick's liberal endowment of yolk can best be appreciated if one compares the course of its development with that of the frog. The yolk in a frog's egg, though considerable in amount, is not sufficient to carry the embryo through all the changes it must undergo before it attains a body organization like that of the parents. By the time all its yolk has been used the frog embryo has grown only to a larval form, commonly called a tadpole, which is still far short of adult structure. This tadpole is able to secure by its own activities sufficient food to complete its growth and to carry it through the remaining developmental steps which bring it out a small frog. The chick has no such interrupted embryology. Its generous supply of yolk is sufficient to provide for a rapid and continuous development so that when it emerges from the shell it is already a miniature of its parents.

Figure 8 shows a section of the hen's ovary which includes several young ova, and one ovum which is nearly ready for liberation. The very young ova lie deeply embedded in the substance of the ovary. As they accumulate more and more deutoplasm they crowd toward the surface and finally project from it, maintaining their connection only by a constricted stalk of ovarian tissue. The protuberance containing the ovum is known as an ovarian follicle. The bulk of the ovum itself is made up of the yolk. Except in the neighborhood of the nucleus the active cytoplasm is but a thin film enveloping the yolk. About the nucleus a considerable mass of cytoplasm is aggregated. The region of the ovum containing the nucleus and the bulk of the active cytoplasm is known as the animal pole because this subsequently becomes the site of greatest protoplasmic activity. The region opposite the animal pole is called the vegetative pole because, while material for growth is drawn from this region, it remains itself relatively inactive.

Enclosing the ovum is a thin non-cellular membrane, the vitelline membrane, which is a secretory product of the cytoplasm of the ovum. Outside the vitelline membrane and very difficult to differentiate from it, is another secreted membrane, the zona radiata, so called because of its delicate radial striations. Immediately peripheral to the zona radiata is an investment of small polygonal cells, the cellular or "granular" zone of the follicle, which is in turn enclosed in a highly vascular coat of connective tissue, the theca folliculi.

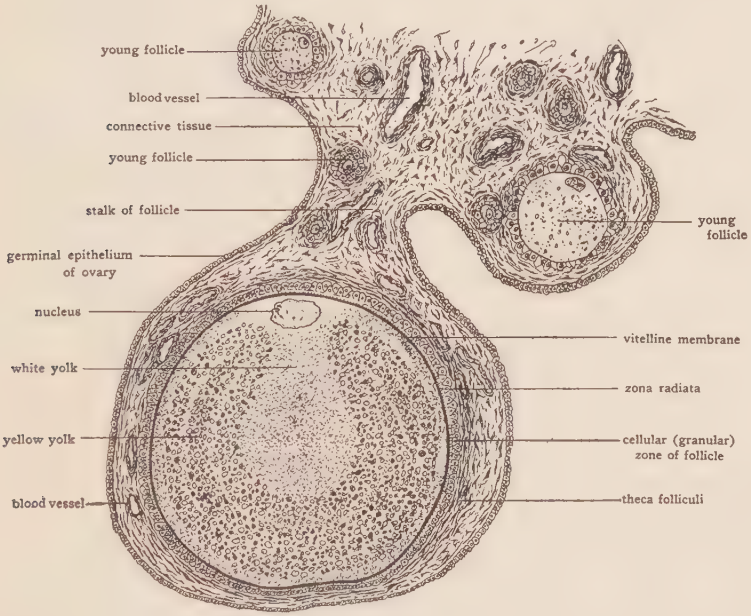


FIG. 8.—Diagram showing the structure of a bird ovum still in the ovary. (*Modified from Lillie, after Patterson.*) The section shows a follicle containing a nearly mature ovum, together with a small area of the adjacent ovarian tissue.

It should be borne in mind that the term ovum, used without qualification, does not carry a precise significance. It refers always to the female sex cell, but it must be specified whether one means a young ovum before all the deutoplasm is accumulated, an ovum with its full complement of deutoplasm but unmatured, or a matured ovum ready for fertilization. The more exact terms which parallel those employed in designating the phases of spermatogenesis avoid this difficulty. The ovum during its period of growth and the accumulation of deutoplasm which we have just been considering is a primary oöcyte,

developmentally homologous to the primary spermatocyte of the male (Fig. 5). The cells of the granular zone of the ovarian follicle are oögonia which have foregone their chances of becoming mature ova. These cells form a protective covering about the oögonium "chosen" for growth into a primary oöcyte and aid in transferring to it the nutriment supplied by the mother from the products of her digested food. This nutritive material is brought in through the blood vessels of the theca, absorbed by the follicular cells, and transferred by them to the growing primary oöcyte where it is elaborated into deutoplasm.

When the full allotment of deutoplasm has accumulated, the investing tissue of the theca is ruptured and the oöcyte is liberated from the ovary. Almost coincidently with ovulation, as the discharge of the ovum is called, the first maturation division occurs. In this the nuclear material of the primary oöcyte is halved between the two resulting cells but one of them gets practically all the cytoplasm and its contained food materials (Fig. 9). Both of these unequal cells are secondary oöcytes, but only the one which received the entire dowry of deutoplasm has any chance of becoming functional. The small oöcyte containing practically no cytoplasm, because it was budded off from the "animal pole" of the ovum, is commonly called a "polar body" or polocyte. This polocyte drags along a discouraged existence for a time and may undergo an abortive second maturation division. But it is doomed to degenerate. Again in the second maturation division all the stored food material goes to one cell. The cell which receives no deutoplasm is called the second polocyte and is, like the first polocyte, destined for degeneration. The cell which has all the deutoplasm that might have been divided among four sister oötids is the mature gamete ready for fertilization (Fig. 9).

If one reviews mentally the phenomena of oögenesis one is impressed both by the underlying parallelism of oögenesis and spermatogenesis and by certain striking differences between the two processes. The early history of the germ plasm is the same in the two sexes, so too is the very definite sequence of gonial cell multiplication, followed by a growth phase, which is in turn followed by two maturation divisions (Fig. 5). But each sex shows certain highly characteristic modifications of the

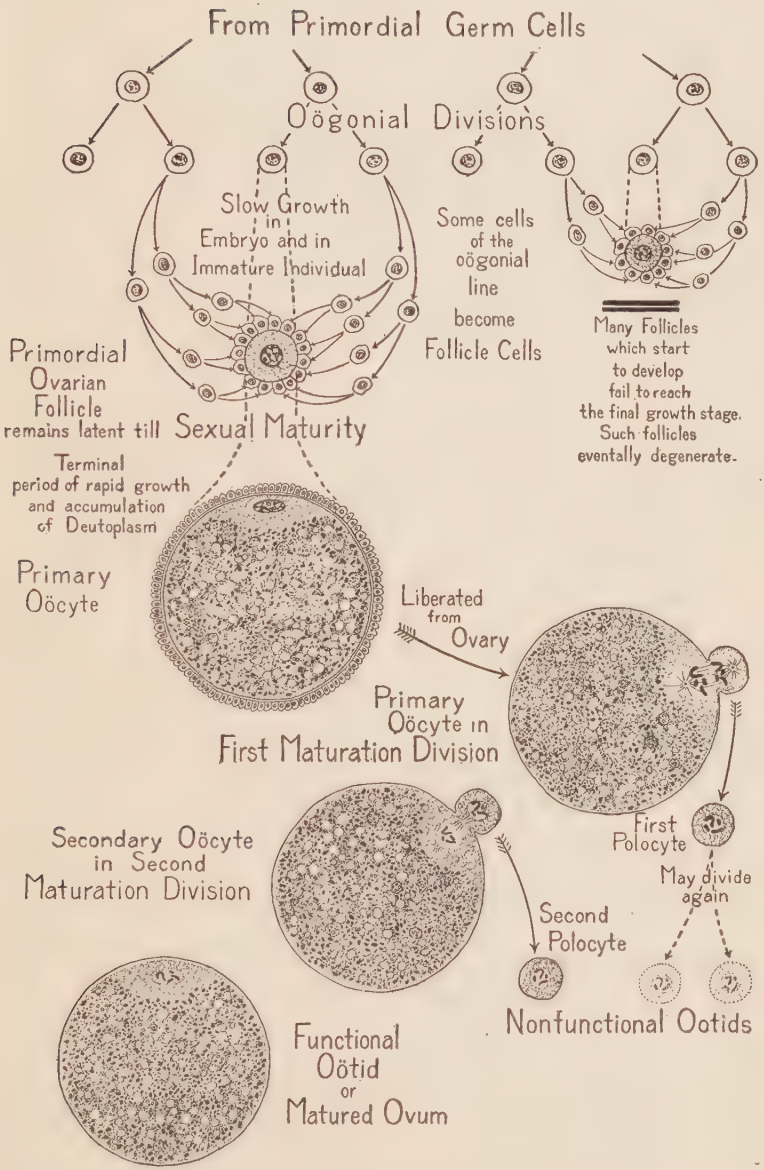

From Primordial Germ Cells
Oögonial Divisions
Slow Growth in Embryo and in Immature Individual
Some cells of the oögonial line become Follicle Cells
Many Follicles which start to develop fail to reach the final growth stage. Such follicles eventally degenerate.
Primordial Ovarian Follicle remains latent till Sexual Maturity
Terminal period of rapid growth and accumulation of Deutoplasm
Primary Oöcyte
Liberated from Ovary
Primary Oöcyte in First Maturation Division
First Polocyte
May divide again
Secondary Oöcyte in Second Maturation Division
Second Polocyte
Nonfunctional Ootids
Functional Oötid or Matured Ovum

FIG. 9.—Diagram to show the main steps in the growth and maturation of the ovum. For the sake of simplicity the species number of chromosomes is assumed to be eight.

process. In the female many potential oögonia are sacrificed in the more elaborate provision for a chosen few, whereas in the male any spermatogonium may produce mature gametes. In the female the growth phase of the primary oöcytes is begun long before sexual maturity, in fact before birth. For, although in some animals there may be new oöcytes formed by oögonial divisions later in life, ordinarily most of the oögonial divisions have occurred during embryonic life, and the ovary at the time of hatching or birth already contains more potential oöcytes than it can endow with deutoplasm and bring to maturity. Thus the growth period by which oögonia are converted into primary oöcytes begins much earlier, lasts much longer, and is quantitatively much greater than the corresponding growth phase of the primary spermatocyte which begins only shortly before sexual maturity. It seems not improbable that this greatly protracted growth phase of the oöcyte may be one of the underlying factors involved in the fact that sterility without obvious cause is more frequently encountered in the female than in the male. Finally, as a result of the two maturation divisions, the oöcyte gives rise to three non-functional polar bodies and but one mature gamete which gets all the food accumulated by the primary oöcyte in its long growth period, whereas each primary spermatocyte produces four functional spermia.

Significance of Maturation.—The events in the maturation of the male and female gametes which have just been discussed are but the more evident phases of the process. There have been changes of profound significance going on at the same time in the nuclear material. It would carry us far afield into cytology and genetics, to discuss these changes in detail and attempt an interpretation of their full meaning. But we can indicate briefly wherein their importance lies.

It has already been stated that the inheritance of an individual comes to it by way of the gametes arising from the germ plasm of its parents. We can be more definite. It comes by way of the chromosomes in the nuclei of the gametes. The chromosomal content of the nuclei in the cells which go to make up the body of an individual is definite and constant. The elaborate mechanism of mitosis splits the chromosomes lengthwise into qualitatively and quantitatively equivalent daughter chromosomes. Thus in each of the countless cell divisions

involved in the growth of an individual the number of chromosomes remains in the daughter cells what it was in the parent cell. The chromosome number so maintained is different in different species, but in the various cells of individuals of the same species it is fixed and definite, the "species number of chromosomes" in the parlance of cytologists and geneticists.

Moreover in each cell division, when the chromosomes take shape from the scattered chromatin granules of the resting nucleus, they reappear in the same characteristic shapes and sizes exhibited by the chromosomes of the previous cell division. This individuality of the chromosomes is very likely to be overlooked by the uninitiated because most schematic diagrams

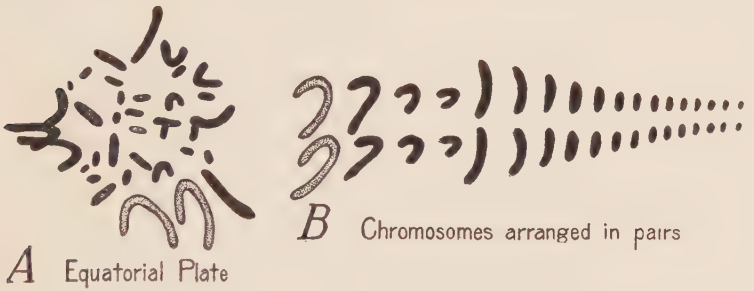


FIG. 10.—The chromosomes of the fowl. (*After Hance, slightly modified as to arrangement.*)

A. Actual appearance (camera lucida drawing, polar view) of the chromosomes as they lie in the equatorial plane of a mitotic spindle just before the beginning of the metaphase splitting.

B. Diagram, to the same scale as *A* showing how the chromosomes may be arranged in pairs, the members of which are similar to each other in size and shape.

illustrating mitosis, for the sake of simplicity, show the chromosomes as if they were all exactly alike. But a cytologist when he has studied an animal intensively will be able to tell us not only how many chromosomes we can count on finding at each mitosis but also how each chromosome will look. One he will characterize as long and bent, another as slender and straight, still another as short and plump, and so on. Furthermore on careful study of these chromosomes one finds that they are present in pairs the members of which are similar in size and shape. The members of a pair are not usually located next to one another on the spindle of an ordinary somatic mitosis, but methodical comparison of their size and shape enables the cytologist to chart the chromosomes of a cell, similar pair, by similar pair (Fig. 10).

The fact that the chromosomes thus occur in recognizable couples is of great significance to the geneticist in his study of the manner in which hereditary traits are passed on from one generation to the next. We are already familiar with the fact that all the cells of an animal's body have been formed by the division and redivision of the fertilized egg-cell or zygote. We know that in each of these mitotic divisions each chromosome is split in half so that the daughter cells receive the same number of chromosomes that the parent cell contained. Obviously then if a cell taken from a rooster's comb or anywhere else in his body showed 36 chromosomes, the zygote from which the rooster grew inevitably had 36 chromosomes. Now if we study the formation of the zygote we find that of its full species number of chromosomes half were brought in by the male gamete and half by the female gamete. And if we study the individual shapes and sizes of their chromosomes we find that each gamete contributed one member of each pair of chromosomes which characteristically appear in the cells of the body in that species.

So when the geneticist finds that a certain chromosome has a knob on its end when an animal he is breeding experimentally shows, let us say red eyes, and lacks this knob in another offspring with white eyes he has a definite physical basis for his assumption that this particular chromosome carries the determining hereditary factor or gene for red eyes. The work of thus tying up specific hereditary traits with definite chromosomes though as yet in its infancy, has already yielded far reaching results in experimental breeding. We are beginning to see more clearly the manner in which both the racial heritage and the particular traits of an individual are passed on. The links in the endless chain of heredity are the chromosomes,—a definite number of pairs maintained constant in all the cells of an individual by mitosis; the same definite number of pairs in the cells of each succeeding generation re-established when each gamete brings in one member of the new pair.

It is in the final two divisions of spermatogenesis and oögenesis, that the chromosomes of the gametes are reduced to half the species number. Cytologists have worked out the mechanism of these maturation divisions with great care in many forms. In some forms chromosome reduction takes place in

the first, in others in the second maturation division. Which of the two divisions is a reduction division in any particular case is of minor significance. The reduction itself is the thing of interest. Stripped of detail and without reference to many peculiar modifications encountered in different animals, a reduction division is a mitosis in which the chromosomes are not split in the metaphase. Instead the chromosomes are redistributed. Half of them go bodily to one daughter cell and half to the other. If the first maturation division is a reduction division the second maturation division is like a typical mitosis with the chromosomes splitting in the metaphase and the daughter cells each receiving the same number of chromosomes as the cell from which they were derived. In contrast to the reduction division this is appropriately called an equation division. Vice versa, if the second maturation division is a reduction division the first is an equation division. Whatever their sequence and whatever the details of the mechanism, the result of the maturation divisions is to halve the species number of chromosomes in the mature gametes of both sexes.

In this halving process the way is paved for separating the chromosomal pairs by a special process termed synapsis which occurs in the prophase of a maturation division but not in an ordinary mitosis. If we look at the spindle of an ordinary mitosis (Fig. 11, *A*) we can recognize the members of the chromosomal pairs by their size and shape, but the members of the pairs do not lie next to one another. When, during the prophase, the spireme thread was broken up into chromosomes these chromosomes aggregated at once in haphazard arrangement at the equator of the spindle. In the prolonged prophase of a reduction division the members of the chromosomal pairs come to lie close to each other and so remain for some time. This pairing off of the chromosomes in the prophase is what is meant by the term synapsis. These synaptic pairs of chromosomes, still in intimate association, finally move to the equator of the spindle (Fig. 11, *D*). When in an ordinary mitosis splitting of the chromosomes would occur, in a reduction division the two members of the synaptic pair are separated from each other, one going bodily to either pole of the spindle (Fig. 11, *E*, *F*). The resulting cells thus receive half the species

number of chromosomes, and this half complement (haploid number) is made up of one member of each of the pairs characteristically present in the species (diploid number). Moreover the cells formed in the reduction division contain different

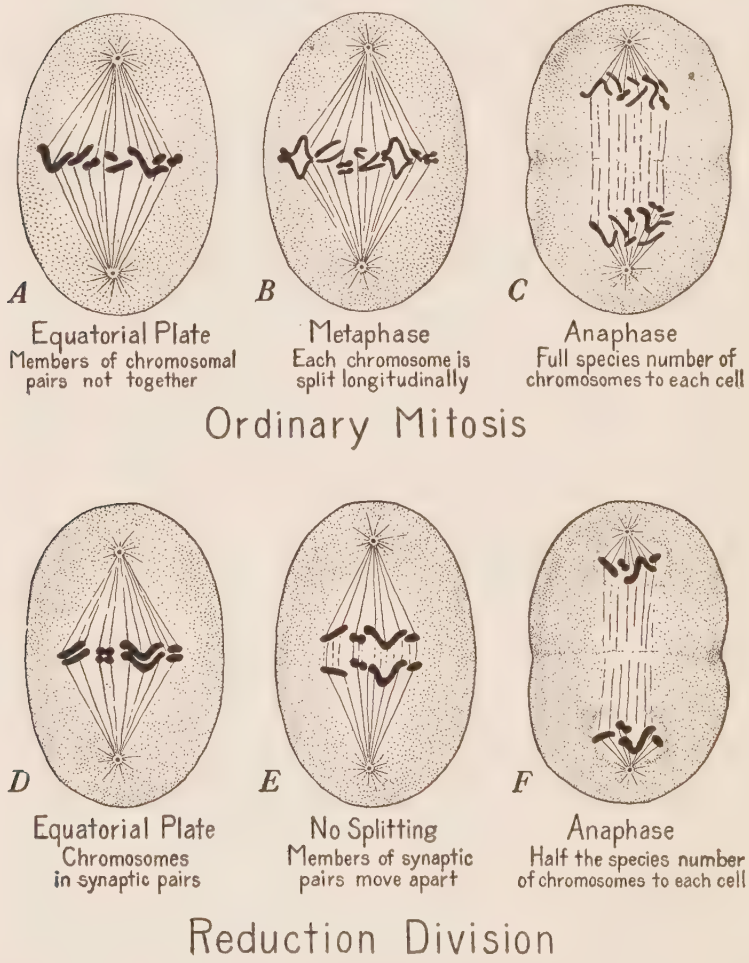


FIG. 11.—Diagrams showing schematically the difference between a reduction division in the process of maturation and an ordinary mitotic division

hereditary potentialities because they contain different chromosomes, not halves of the same chromosomes as results in an ordinary mitosis. What hereditary possibilities are discarded into the polar bodies thrown off from the female gamete and what retained in the mature ovum is a matter of chance distri-

bution. What potentialities find their way into the particular sperm which alone, out of millions of its fellows, fertilizes the ovum is likewise fortuitous.

"Thus in the game of life, the maturation processes virtually shuffle the hereditary pack and deal out half a 'hand' to each gamete. A full hand is obtained by drawing a partner from the 'board,'—by combining with some other gamete of the opposite sex. Hence offspring resemble their parents because they play the game of life with the same kind of cards, but not, however, with the same hands. The minor differences in offspring, or the variations from the standard type that always go with these basic resemblances, are due to variations in the distribution of the genes during maturation" and new combinations made in fertilization.

Thus there is produced sufficient stability to insure continuity and at the same time sufficient variety to insure progress. "For the offspring will in the main resemble progenitors which have successfully lived in the prevailing conditions of the past, but will exhibit sufficient variability among themselves to insure that some of them shall successfully live in any conditions likely to arise in the future."[1]

Sex Determination.—Innumerable theories purporting to explain what determines the sex of an individual have been advanced only to be discredited. Under such circumstances one hesitates to tread this still uncertain ground. There are current, however, so many absurd and groundless ideas on the subject that it might be well to indicate in what direction our present information points.

Reference has already been made to the fact that cytologists and geneticists have begun to correlate peculiarities of individual chromosomes with bodily characteristics. Apparently sex also is influenced by the particular chromosomal combination which occurs with the fusion of the two gametes. Nearly all the animals which have been critically studied show a slight but strikingly consistent difference in tne chromosome picture exhibited by the cells in the bodies and in the germ line of the two sexes. In one sex, the cells have all their pairs of chromosomes symmetrically mated. In the opposite sex one of the pairs of chromosomes is likely to be peculiar in that

[1] From Wm. Patten. "Life, Heredity and Evolution."

its members are quite different in size and shape instead of being like each other as is the general rule. In some cases one member of this pair seems to be altogether missing. After the manner of a mathematician in calling the "unknowns" in his problem by the non-committal names of X and Y, when this peculiar condition was discovered the members of this unmatched pair of chromosomes were designated as X and Y, or if one member was missing, X and O. Subsequent breeding experiments have given us considerable evidence indicating that the reason the members of the X-Y pair are unlike is because something that makes for femaleness is carried in the X-chromosome and absent in the Y.

Now we know that in the reduction division of the gametes the members of the chromosomal pairs are separated (Fig. 11). If the X-chromosome does carry whatever makes for femaleness and its Y-mate lacks this or carries an antagonistic male determining factor, it is evident that when in a reduction division the members of this chromosomal couple go to opposite poles of the spindle the resulting cells will carry opposite sex tendencies (Fig. 12).

Assuming (as actually is the case in most of the forms so far studied[1]) that it is the female which has all its chromosomes symmetrically paired and the male that has an unbalanced pair, the chromosomal combinations taking place in fertilization can readily be depicted graphically (Fig. 13). When the female gametes mature all of them will appear alike as far as chromosomes are concerned because in maturation one member of each balanced pair goes to each cell (Fig. 11, *D-F*). The reduction division in spermatogenesis, however, separates the unbalanced X-Y pair and consequently half the gametes receive an X chromosome and the other half a Y chromosome (Fig. 12). The chances of an X-carrying gamete uniting with an ovum and giving rise to a female are the same as those of a Y-bearing chromosome fertilizing the ovum to produce a male

[1] Recent investigations indicate that the fowl probably is one of the exceptions to the general rule that it is the male which produces two types of gametes or, to use the technical expression, exhibits digamety. Apparently the hen produces ova which differ in sex determining potentialities while the cock produces spermia which are all alike in this respect. The interpretation given in the text can readily be inverted to cover female digamety although it is outlined concretely for the more usual condition of male digamety.

(Fig. 13). Thus this theory accounts at the same time for the approximate equality of the numbers of males and females produced and for the observed differences in the chromosome

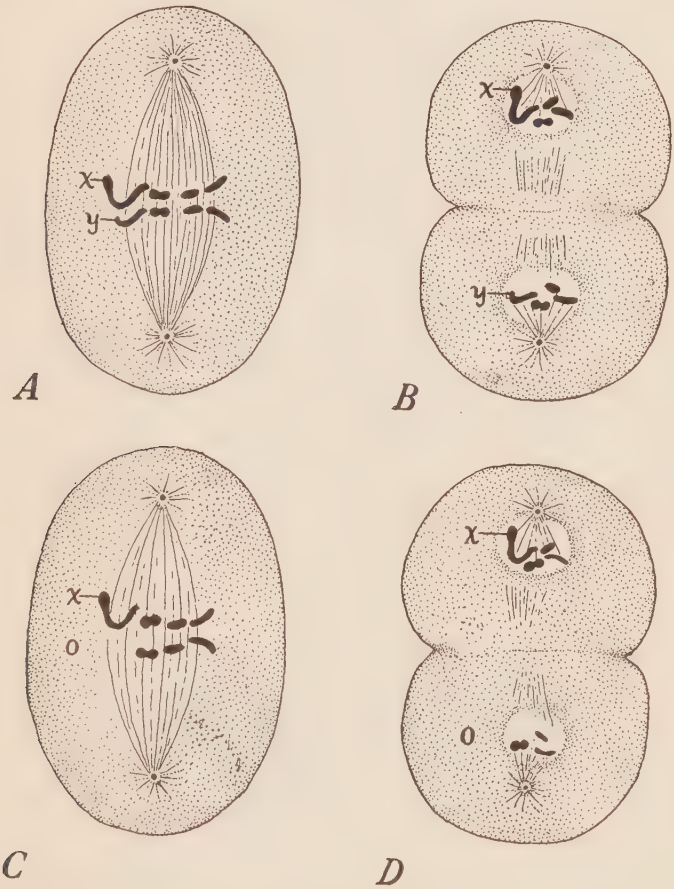


FIG. 12.—Schematic diagrams indicating the manner in which the chromosomes believed to be associated with sex determination are distributed in the reduction division. *A* and *B* show the process for an X-Y combination; *C* and *D* for an X-O combination. In either case the factor for femaleness is supposed to be carried in the X-chromosome.

picture presented by the two sexes. Recent experimental evidence seems to indicate that there are other factors involved in sex determination, and that sex may not be as irrevocably fixed by the chromosomal combination as was at first believed. Nevertheless it seems to be quite clearly established that these

chromosomal differences constitute an important, probably the major, factor in the determination of sex.

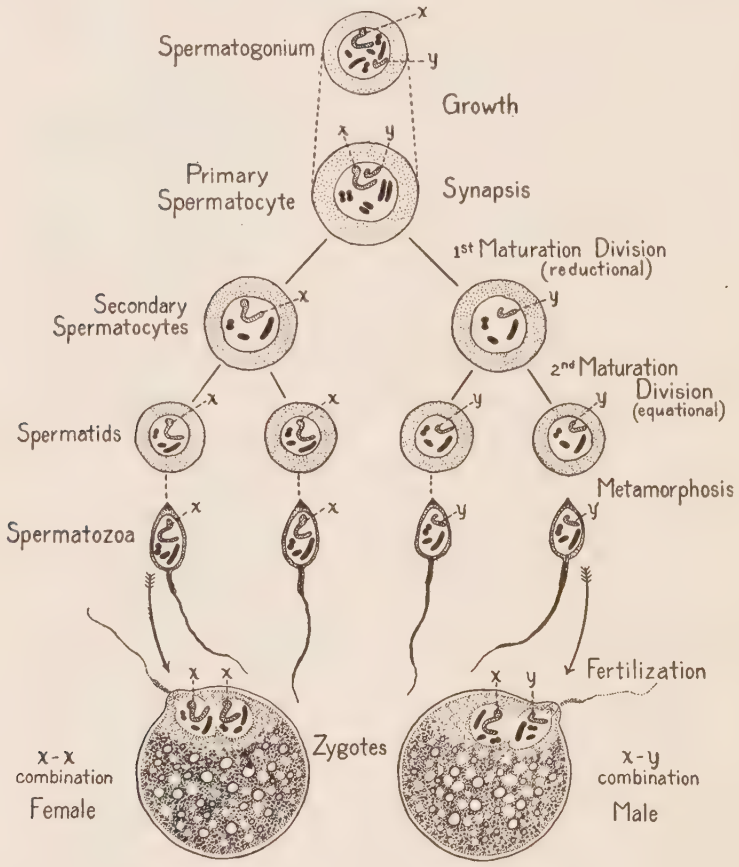


FIG. 13.—Schematic diagram showing the separation of the members of the sex chromosome pair in maturation, and their recombinations in fertilization. It is assumed that the species number of chromosomes is eight and that it is the male which produces gametes of different potentialities with regard to sex determination. The sex chromosomes are stippled; other chromosomes are drawn in solid black.

Fertilization.—Direct observations of fertilization in such forms as birds and mammals where the process occurs inside the body of the female are exceedingly fragmentary. Fortunately much more information is available from the study of water-living animals in which the eggs are commonly deposited prior to fertilization and fertilized in the water outside the

maternal body. When such eggs are placed in a watch glass and sperm introduced the whole process of fertilization can be studied under the microscope. Interpreting in the light of such observations we can piece together a generalized account of the process of fertilization which in all probability is applicable without essential modification to birds or mammals.

In the testis and sperm ducts the spermatozoa show but little indication of their power of locomotion. During coitus, however, certain accessory glands opening into the sperm ducts discharge a considerable amount of fluid in which the spermia are floated and in which they become actively motile. When this fluid has been deposited in the genital tract of the female, the spermia are under conditions not unlike the spermia of a fish discharged into sea water near eggs recently laid by the female. The bird ovum, recently liberated from the ovary, is awaiting the spermia in the upper part of the oviduct. Here the ovum becomes surrounded by swarms of spermatozoa which have made their way thither by their own active locomotion through the lower part of the genital tract.

The process to this point is known as insemination. Still other preliminary steps are to be passed through before the nuclei of the male and female gametes unite to complete the process of fertilization. The spermia once having reached the neighborhood of the ovum tend to remain there held by some chemical interaction which is not as yet fully understood. Soon a minute cone of ovarian cytoplasm rises up to meet one of the spermia and draws it into the ovum (Fig. 14, *A*, *B*). Not infrequently, especially in birds, several spermatozoa penetrate the ovum, but since only one of these takes part in fertilization we can neglect the others. Once a sperm has penetrated the ovum the peripheral cytoplasm produces a clear viscid substance which adheres to the inner face of the vitelline membrane thickening it into the so-called "fertilization membrane." The fact that no spermia penetrate the ovum after the fertilization membrane has been formed may be due as much to chemical changes in the ovum following the entrance of the sperm as to the mechanical impediment offered by the membrane.

The first maturation division of the ovum has usually occurred at about the time of ovulation. The second matura-

tion division is very likely not to occur until after the ovum has been penetrated by a spermium. While this final maturation division is being completed by the ovum the spermium undergoes very striking changes in appearance. Its tail is usually dropped off when it enters the ovum. Once within the ovarian cytoplasm the sperm head increases rapidly in size and its chromosomal contents again become distinctly recognizable (Fig. 14, *B-D*). In this condition it is called the male

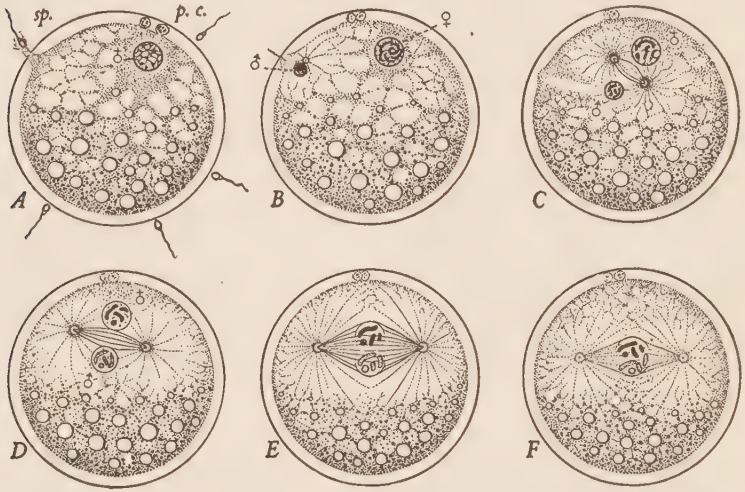


FIG. 14.—Diagram illustrating schematically the process of fertilization and the formation of the first cleavage spindle. (*After Wm. Patten.*) The species number of chromosomes is assumed to be eight. The chromosomes carried by the ovum are shown in solid black; those brought in by the spermium are differentiated by stippling.

ABBREVIATIONS

sp., spermium p.c., polocyte (polar body).
The male and female symbols designate the male and female pronuclei respectively.

pronucleus, and the nucleus of the ovum after the second maturation division has been completed is called the female pronucleus. Meanwhile the centrosomal apparatus, which was carried in the spermium where its tail was attached to its head, becomes much more conspicuous. As it approaches the female pronucleus, the male pronucleus with its associated centrosome rotates so that the centrosome moves ahead of the chromatin material (Fig. 14, *B*, *C*). By the time the two pronuclei are close to each other this centrosome has divided and formed a mitotic spindle on which the chromosomes brought

in by the sperm and those in the ovum both aggregate (Fig. 14, *E*, *F*). Fertilization can be regarded as complete when the maternal and paternal chromosomes are thus grouped together ready to be split in the impending first cell division in the life of the new individual.

The Formation of the Accessory Coverings of the Ovum. Fertilization, as have seen, normally takes place just as the ovum is entering the oviduct. The accessory coverings, as the albumen, shell membrane and shell are called, are secreted about the ovum during its subsequent passage toward the cloaca. In the part of the oviduct adjacent to the ovary a mass of stringy albuminous material is produced. This adheres closely to the vitelline membrane and projects beyond it in two masses extending in either direction along the oviduct. Due to the spirally arranged folds in the walls of the oviduct, the egg as it moves toward the cloaca is rotated. This rotation twists the adherent albumen into the form of spiral strands projecting at either end of the yolk, known as the chalazæ (Fig. 15). Additional albumen, which has been secreted abundantly in advance of the ovum by the glandular lining of the oviduct, is caught in the chalazæ and during the further descent of the ovum is wrapped about it in concentric layers. These lamellæ of albumen may be easily demonstrated in an egg which has had the albumen coagulated by boiling. The albumen-secreting region of the oviduct constitutes about one-half of its entire length.

The shell membranes which consist of sheets of matted organic fibers are added farther along in the oviduct. The shell is secreted as the egg is passing through the shell-gland portion of the oviduct. The entire passage of the ovum from the time of its discharge from the ovary to the time when it is ready for laying has been estimated to occupy about 22 hours. If the completely formed egg reaches the cloacal end of the oviduct during the middle of the day it is usually laid at once, otherwise it is likely to be retained until the following day. This over-night retention of the egg is one of the factors which accounts for the variability in the stage of development reached at the time of laying.

The Structure of the Egg at the Time of Laying.—The arrangement of structures in the egg at the time of laying is

shown in Figure 15. Most of the gross relationships are already familiar because they appear so clearly in eggs which have been boiled. If a newly laid egg is allowed to float free in water until it comes to rest and is then opened by cutting away the part of the shell which lies uppermost, a circular whitish area will be seen to lie atop the yolk. In eggs which have been fertilized this area is somewhat different in appearance and noticeably larger than it is in unfertilized eggs. The differences are due to the development which has taken place in fertilized eggs during their passage through the oviduct. The aggrega-

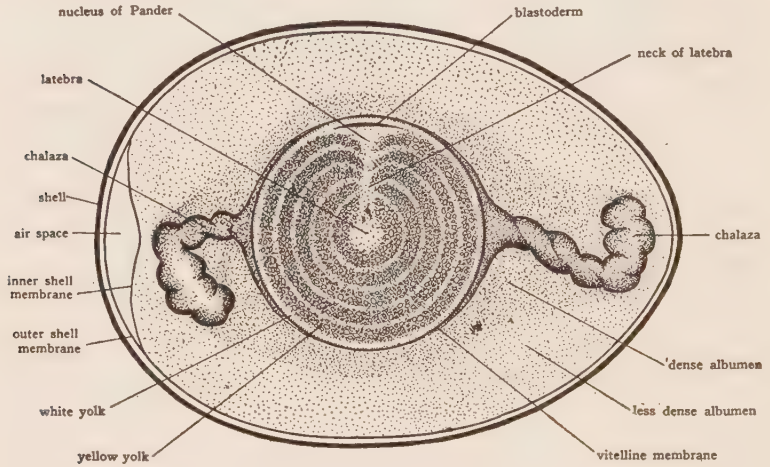


FIG. 15.—Diagram of the hen's egg in longitudinal section. (*After Lillie.*) The relations of the various parts of the egg at the time of laying are indicated schematically.

tion of cells which in fertilized eggs lies in this area is known as the blastoderm. The structure of the blastoderm and the manner in which it grows will be taken up in the next chapter.

Close examination of the yolk will show that it is not uniform throughout either in color or in texture. Two kinds of yolk can be differentiated, white yolk, and yellow yolk. Aside from the difference in color visible to the unaided eye, microscopical examination will show that there are differences in the granules and globules of the two types of yolk, those in the white yolk being in general smaller and less uniform in appearance. The principal accumulation of white yolk lies in a central flask-shaped area, the latebra, which extends toward the blastoderm and flares out under it into a mass known as the nucleus of

Pander. In addition to the latebra and the nucleus of Pander there are thin concentric layers of white yolk between which lie much thicker layers of yellow yolk. The concentric layers of white and yellow yolk are said to indicate the daily accumulation of deutoplasm during the final stages in the formation of the egg. The outermost yolk immediately under the vitelline membrane is always of the white variety.

The albumen, except for the chalazæ, is nearly homogeneous in appearance, but near the yolk it is somewhat more dense than it is peripherally. The chalazæ serve to suspend the yolk in the albumen.

The two layers of shell membrane lie in contact everywhere except at the large end of the egg where the inner and outer membranes are separated to form an air chamber. This space is stated (Kaupp) to appear only after the egg has been laid and cooled from the body temperature of the hen (about 106°F.) to the ordinary temperatures. In eggs which have been kept for any length of time the air space increases in size due to evaporation of part of the water content of the egg. This fact is taken advantage of in the familiar method of testing the freshness of eggs by "floating them."

The egg shell is composed largely of calcareous salts. These salts are derived from the food of the mother and if lime-containing substances are not furnished in her diet the shell is defectively formed or even altogether wanting. The shell is porous allowing the embryo to carry on exchange of gases with the outside air by means of specialized vascular membranes arising in connection with the embryo but lying outside it, directly beneath the shell.

Incubation.—When an egg has been laid, development ceases unless the temperature of the egg is kept nearly up to the body temperature of the mother. Moderate cooling of the egg does not, however, result in the death of the embryo. It may resume its development if it is brooded by the hen or artificially incubated even after the egg has been kept for many days at ordinary temperatures.

The normal incubation temperature is that at which the egg is maintained by the body heat from the brood-hen. This is somewhat below the blood heat of the hen (106°F.). When an egg is allowed to remain undisturbed the yolk rotates so that

the developing embryo lies uppermost. Its position is then such that it gets the full benefit of the warmth of the mother.

In incubating eggs artificially the incubators are usually regulated for a heat of 100°–101°F. (37°–38°C.). At this temperature the chick is ready for hatching on the twenty-first day. Development will go on at considerably lower temperatures but its rate is retarded in proportion to the lowering of the temperature. Below about 21 degrees Centigrade development ceases altogether.

If eggs which have been cooled after laying are to be incubated for the purpose of securing embryos of a particular stage of development, three or four hours are ordinarily allowed for the egg to become warmed to the point at which development begins again. For example if an embryo of "24-hours incubation age" is desired the egg should be allowed to remain in the incubator about 27 hours. Even with allowance made for the warming of the egg and with exact regulation of the temperature of the incubator, the stage of development attained in a given incubation time will vary widely in different eggs. The factor of individual variability, which must always be reckoned with in developmental processes, undoubtedly accounts for some of the variation. The different time occupied by different eggs in traversing the oviduct, the over-night retention of eggs not ready for laying till toward sundown, and especially the varying time different eggs have been brooded before being removed from the nest, account for further variations. The designation of the age of chicks in hours of incubation is, therefore, not exact, but merely a convenient approximation of the average condition reached in that incubation time.

CHAPTER III

THE PROCESS OF SEGMENTATION

THE EFFECT OF YOLK ON SEGMENTATION; THE SEQUENCE AND ORIENTATION OF THE CLEAVAGE DIVISIONS IN BIRDS.

The Effect of Yolk on Segmentation.—Immediately after its fertilization the ovum enters upon a series of mitotic divisions which occur in close succession. This series of divisions constitutes the process of segmentation or cleavage. In birds segmentation takes place before the egg is laid, during the time it is traversing the oviduct.

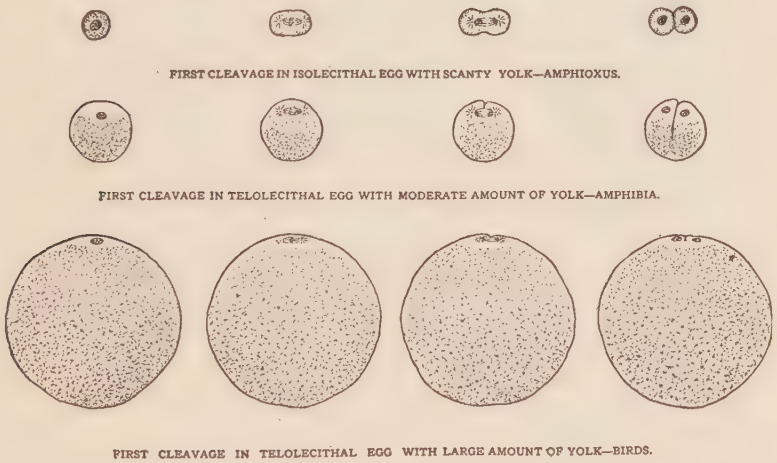

FIRST CLEAVAGE IN ISOLECITHAL EGG WITH SCANTY YOLK—AMPHIOXUS.
FIRST CLEAVAGE IN TELOLECITHAL EGG WITH MODERATE AMOUNT OF YOLK—AMPHIBIA.
FIRST CLEAVAGE IN TELOLECITHAL EGG WITH LARGE AMOUNT OF YOLK—BIRDS.

FIG. 16.—Schematic diagrams to indicate the effect of yolk on the first cleavage division.

A mitotic division, whether it be a cleavage division of the ovum or the division of some other cell, is carried out by the active protoplasm of the cell. The food material stored in an egg cell as deutoplasm is non-living and inert. The deutoplasm has no part in mitosis except as its mass mechanically influences the activities of the protoplasm of the cell. It is obvious that any considerable amount of yolk will retard the division, or

prevent the complete division, of the fertilized ovum. The amount and distribution of the yolk will therefore determine the type of segmentation.

Figure 16 shows diagrammatically the manner in which the first cleavage division is carried out in three types of eggs having different relative amounts and different distributions of yolk and protoplasm. In the egg of Amphioxus the yolk is relatively meager in amount and fairly uniformly distributed throughout the cell. An ovum with such a yolk distribution is

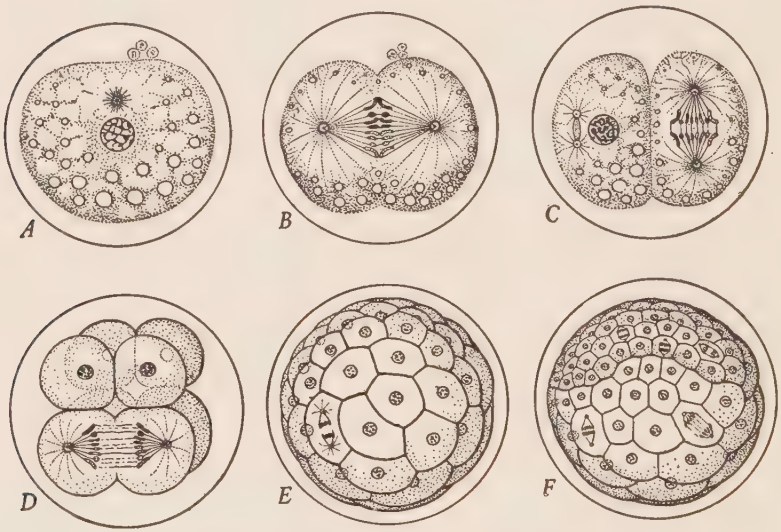


FIG. 17.—Cleavage in an egg having but a small amount of yolk in the cytoplasm. (*After Wm. Patten.*) Note that the blastomeres are separated from each other promptly and completely.

termed isolecithal (homolecithal). An isolecithal egg undergoes a type of cleavage which is essentially an unmodified mitosis. The yolk is not sufficient in amount, nor sufficiently localized to alter the usual mode of cell division.

In Amphibia the ovum contains a considerable amount of yolk and the accumulation of the yolk at one pole has crowded the nucleus and active cytoplasm of the ovum toward the opposite pole. An egg in which the yolk is thus concentrated at one pole is termed telolecithal. Cleavage in such an egg is initiated at the animal pole where the nucleus and most of the active cytoplasm are located. The division of the nucleus is a typical mitotic division. The division of the cytoplasm is

effected rapidly at the animal pole of the egg where the active cytoplasm is aggregated. When, however, the yolk mass is encountered, the process is greatly retarded. So slowly, in fact, is the division of the yolk accomplished, that succeeding cell divisions begin at the animal pole of the egg before the first cleavage is completed at the vegetative pole. This tendency for cell divisions to follow each other in rapid succession is met

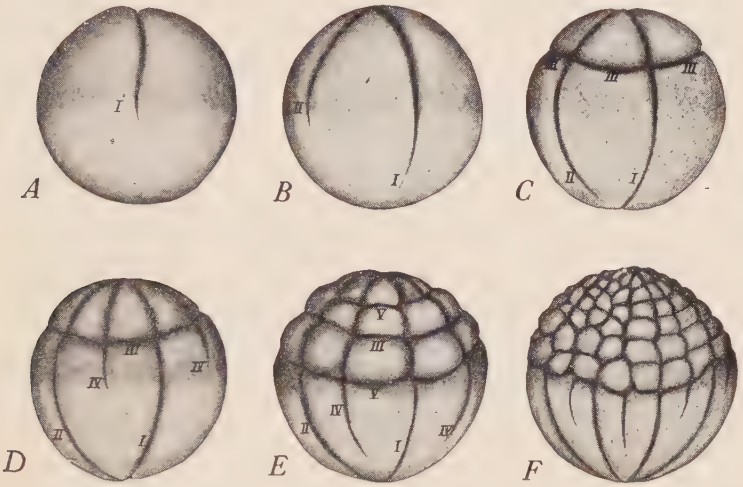


FIG. 18.—Cleavage in the frog's egg. The darkness of the upper hemisphere is due to the presence of pigment in the cytoplasmic cap, while the lighter appearance of the lower hemisphere is caused by the massing of yolk in that part of the egg. The cleavage furrows are designated by Roman numerals indicating the order of their appearance. In *A* and *B* note the retarding effect of the yolk on the extension of the cleavage furrows toward the vegetative pole. In *C* observe the displacement of the third cleavage furrow away from the yolk-laden pole, toward the center of the mass of active cytoplasm. The displacement of the center of activity from the geometrical center of the egg, and the mechanical retardation of cleavage at the vegetative pole,—both due to the yolk mass,—result in the formation of a morula with many small blastomeres in the animal (apical) hemisphere and fewer large blastomeres in the vegetative (abapical) hemisphere.

with in all types of cleavage regardless of the amount of yolk present. Thus the resultant cells do not have time to grow to the size of their parent cells and, paradoxically, the growing embryo for a time shows smaller and smaller cells (Fig. 17). When, as for example in the egg of a frog, the yolk impedes division at the vegetative pole, the cells of this hemisphere remain much larger than those at the animal pole because in a given time they have not divided as often (Fig. 18). Later we shall see that the moulding effect of the yolk on embryonic

configuration is by no means limited to the period of cleavage but, on the contrary, that its effects are manifest well into the period of organ formation. Meanwhile the obvious modifying effect on cleavage exerted by the moderate amount of yolk present in the frog's egg indicates the profound effect we can expect the much greater yolk-mass of the hen's egg to have on the course of chick development.

The Sequence and Orientation of the Cleavage Divisions in Birds.—Cleavage in bird's eggs begins as it does in the eggs of Amphibia (Fig. 16), but the mass of the inert yolk material is so great that the yolk is not divided. The process of segmentation is limited to the small disc of protoplasm lying on the surface of the yolk at the animal pole, and is for this reason referred to as discoidal cleavage (Fig. 19). The fact that the whole egg is not divided is indicated by designating the process as partial (meroblastic) cleavage in distinction to the complete cleavage (holoblastic) seen in eggs containing less yolk. The cells formed in the process of segmentation are known as blastomeres whether they are completely separated, as is the case in holoblastic cleavage, or only partially separated, as in meroblastic cleavage.

In the egg of a bird which is about to undergo cleavage, the disc of active protoplasm at the animal pole (blastodisc) is a whitish, circular area about three millimeters in diameter. The central portion of the blastodisc is surrounded by a somewhat darker appearing marginal area known as the periblast. The protoplasm of the blastodisc, especially in the periblast region, blends into the underlying white yolk so that it is difficult to make out any line of demarcation between the two. It is in the central area of the blastodisc that cleavage furrows first appear. Neither the nuclei resulting from the early cleavages nor the cleavage furrows invade the marginal periblast until very late in the process of segmentation.

The nature of the series of divisions in the meroblastic, discoidal cleavage characteristic of the eggs of birds is, as has already been pointed out, predetermined to a great extent by the amount and distribution of the yolk. Another determining factor is the tendency of mitotic spindles to develop so that the long axis of the spindle lies in the axis of the greatest dimension of the unmodified cytoplasm. The cleavage furrow always

forms at right angles to the long axis of the mitotic spindle. Figure 19 shows the succession of the cleavage divisions in the egg of the pigeon. The diagrams represent surface views of the blastodisc and an area of the surrounding yolk, the shell and albumen having been removed. The observer is looking directly at the animal pole. Figure 19, *A*, should be compared with figure 16 which shows sections cut in a plane passing vertically through the blastodisc at right angles to the plane of the first cleavage. The first cleavage furrow (Fig. 19, *A*, *I-I*) cuts into the egg in a plane coinciding with the axis passing through the animal pole and the vegetative pole. The two daughter cells or blastomeres resulting from the first cleavage are not completely walled off but each remains unseparated from the underlying yolk (Fig. 16).

In each of the two blastomeres resulting from the first cleavage division, mitotic spindles initiating the second cleavage arise at right angles to the position which was occupied by the first cleavage spindle (Fig. 20, cf. *A* and *B*). This determines that the two simultaneously appearing second cleavage furrows will be at right angles to the first. Since these two second cleavage furrows lie in the same plane and are apparently continuous they are usually considered together. A very good way of acquiring a clear conception of the orientation of the cleavage planes is to cut them in an apple. Let the core of the apple represent the animal-vegetative axis of the egg. The first cleavage furrow can be represented by notching the apple lengthwise, that is as one ordinarily starts to split an apple into halves. The second cleavage furrow can be represented by cutting into the apple again in a plane passing through the axis of the core, but at right angles to the first cut, as one would start to quarter the apple.

The third cleavage furrows are variable in number and in position. In the most typical cases each of the four blastomeres established by the first two cleavages divides again so that eight blastomeres are formed (Fig. 19, *C*). Frequently, however, the third cleavage appears at first in only two of the blastomeres so that six cells result instead of eight.

The fourth series of cleavages takes place in such a manner that the central (apical) ends of the eight cells established by the third cleavage are cut off from their peripheral portions.

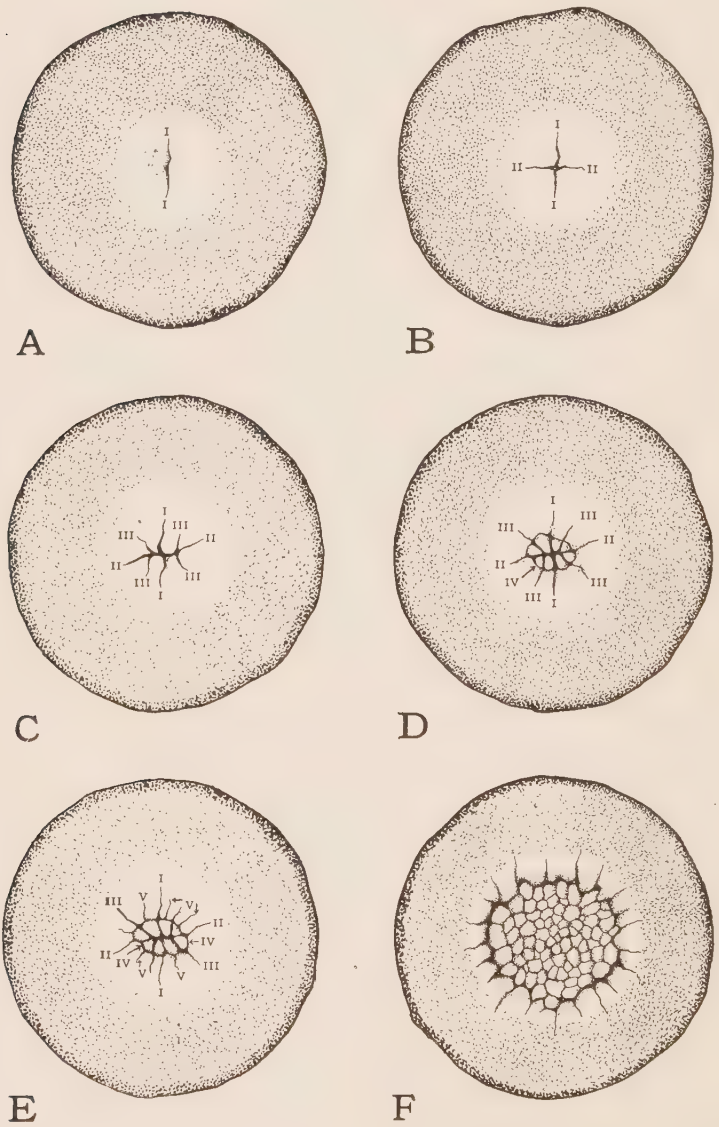


FIG. 19.—Surface aspect of blastoderm at various stages of cleavage. (*Based on Blount's photomicrographs of the pigeon's egg.*) The blastoderm and the immediately surrounding yolk are viewed directly from the animal pole, the shell and albumen having been removed. The order in which the cleavage furrows have appeared is indicated on the diagrams by Roman numerals.

A, first cleavage; *B*, second cleavage; *C*, third cleavage; *D*, fourth cleavage; *E*, fifth cleavage; *F*, early morula.

The combined contour of the fourth cleavage furrows forms a small irregularly circular furrow the center of which is the point at which the first two cleavage planes intersect (Fig. 19, *D*). The central cells now appear completely separated in a surface

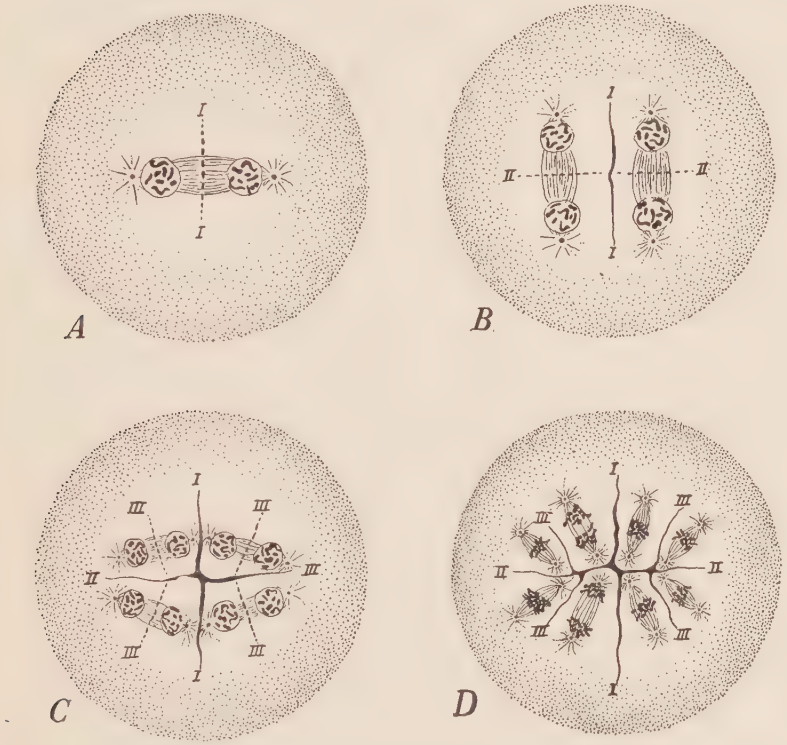


FIG. 20.—Schematic diagrams of cleavage in the bird's egg, indicating the manner in which mitotic spindles become established with their long-axis coinciding with the long-axis of cytoplasmic mass. Following the division of the chromosomes, the cleavage furrow dividing the cytoplasm appears in the equatorial plane of the spindle, *i.e.* at right angles to the axis of the spindle.

A, first cleavage furrow appearing at dotted line I-I; *B*, first cleavage furrow clearly marked and second appearing along dotted line II-II; *C*, third cleavage furrows forming; *D*, fourth cleavage spindles formed; cleavage furrows will appear later in positions indicated in figure 19, *D*.

view of the blastoderm, but sections show them still unseparated from the underlying yolk.

After the fourth, the succession of cleavages becomes irregular. In surface view it is possible to make out cleavage furrows that divide off additional "apical" cells (*i.e.* cells located centrally in the blastoderm and consequently at the apical or animal pole of the egg) and other, radial furrows that further divide

the peripheral cells. Figures 19, *E* and *F*, show the increase in number of cells and their extension out over the surface of the yolk, resulting from the succession of cleavages. When the process of segmentation has progressed to the stage in which the cleavage planes are irregularly placed and the number of cells considerable, the term blastoderm is applied to the entire group of blastomeres formed by the segmentation of the blastodisc.[1]

In addition to the cleavages which are indicated on the surface, vertical sections of the 32-cell embryo show cleavage planes of an entirely different character. These cleavages appear below the surface and parallel to it. They establish a superficial layer of cells which are completely delimited. These superficial cells rest upon a layer of cells which are continuous on their deep faces with the yolk. Continued divisions of the same type eventually establish several strata of superficial cells. This process appears first in the central portion of the blastoderm. It progresses centrifugally as the blastoderm increases in size but does not extend to its extreme margin. The peripheral margin of the blastoderm remains a single cell in thickness and the cells there lie unseparated from the yolk.

[1] While but a single spermatozoön takes part in fertilization other spermatozoa become lodged in the cytoplasm of the blastodisc. The nuclei of these spermatozoa migrate to the peripheral part of the blastoderm where they are recognizable for some time as the so-called accessory sperm nuclei. Some of them appear to undergo divisions which are accompanied by slight indications of division in the adjacent cytoplasm. The short superficial grooves thus formed are termed accessory cleavage furrows. No cells are formed by the accessory "cleavages." The sperm nuclei soon degenerate, the superficial furrows fade out, and usually as early as the 32-cell stage all traces of the process have disappeared without, as far as is known, affecting in any way the development of the embryo.

CHAPTER IV

THE ESTABLISHMENT OF THE ENTODERM

THE MORULA STAGE; THE FORMATION OF THE BLASTULA; THE EFFECT OF YOLK ON GASTRULATION; GASTRULATION IN BIRDS.

The Morula Stage.—It should by no means be inferred that cell division ceases with the cleavage divisions. The end of the "segmentation stage" is not marked by even a retardation in the succession of mitoses. Segmentation as a phase of development is regarded as ending when the progress of events ceases to be indicated merely by increase in the number of cells, and begins to involve the localized aggregation and differentiation of various groups of cells. Development progresses from phase to phase without abrupt change or interruption. The nomenclature and limitation of the various periods of development are largely arbitrary and the use of terms designating embryological stages should not be allowed to obscure the fact that the whole process is continuous.

In eggs without a large amount of yolk, segmentation results in the formation of a rounded, closely packed mass of blastomeres (Fig. 17). This is known as a morula from its resemblance to the mulberry fruit which is in form much like the more familiar raspberry or blackberry. At the end of segmentation the chick embryo has arrived at a stage which corresponds with the morula stage of forms with less yolk. It consists of a disc-shaped mass of cells several strata in thickness (the blastoderm) lying closely applied to the yolk. In the center of the blastoderm the cells are smaller and completely defined; at the periphery the cells are flattened, larger in surface extent, and are not walled off from the yolk beneath.

The Formation of the Blastula.—The morula condition is of short duration. Almost as soon as it is established there begins a rearrangement of the cells presaging the formation of the blastula. A cavity is formed beneath the blastoderm by the

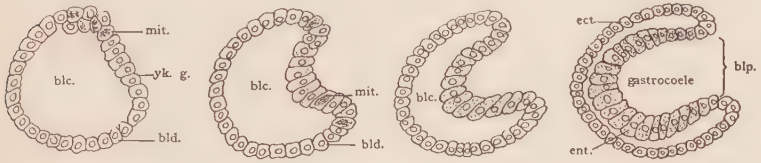
detachment of its central cells from the underlying yolk while the peripheral cells remain attached. The space thus established between the blastoderm and the yolk is termed the segmentation cavity (blastocœle). The marginal area of the blastoderm in which the cells remain undetached from the yolk and closely adherent to it, is called the zone of junction. With the establishment of the blastocœle the embryo is said to have progressed from the morula to the blastula stage.

Figure 22, *D*, shows the conditions seen on sectioning the blastula of a bird. Only the blastoderm and the immediately underlying yolk are included in the diagram. At this magnification the complete yolk must be imagined as about three feet in diameter. The structure of the bird embryo in these stages may be brought in line with the morula and blastula stages of forms having little yolk, if the full significance of the great yolk mass is appreciated. Instead of being free to aggregate first into a solid sphere of cells (morula) and then into a hollow sphere of cells (blastula), as takes place in forms with little yolk, the blastomeres in the bird embryo are forced to grow on the surface of a large yolk sphere. Under such mechanical conditions the blastomeres are forced to become arranged in a disc-shaped mass on the surface of the yolk. If one imagines the yolk of the bird morula removed, and the disc of cells left free to assume the spherical shape dictated by surface tension, its comparability with the morula in a form having little yolk becomes apparent.

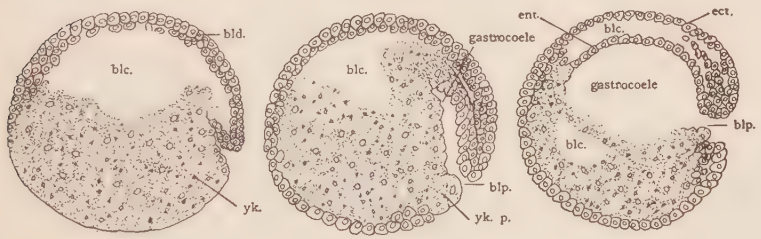
The process of blastulation also is modified by the presence of a large amount of yolk. There can be no simple hollow sphere formation by rearrangement of the cells if the great bulk of the morula is inert yolk. But the cells of the central region of the blastoderm are nevertheless separated from the yolk to form a small blastocœle. The yolk constitutes the floor of the blastocœle and at the same time by reason of its great mass nearly obliterates it. If we imagine the yolk removed from the blastula and the edges of the blastoderm pulled together the chick blastula approaches the form of the blastula in embryos with little yolk.

The Effect of Yolk on Gastrulation.—The process of gastrulation begins as soon as blastulation is accomplished. With the appearance of this new process we think of the embryo as

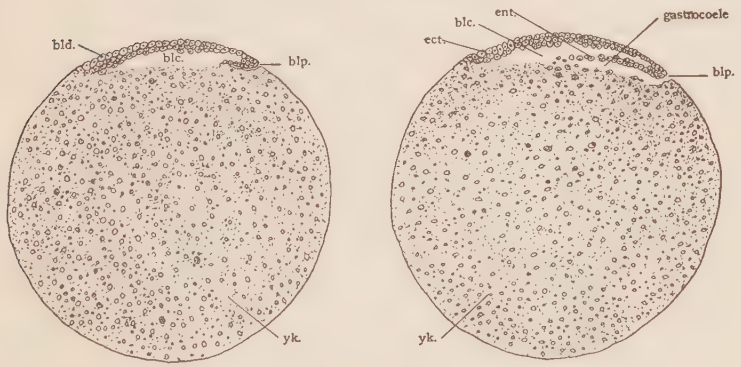
progressing into the next phase of its development, commonly called the "gastrula stage" (diminutive from Greek word *gaster* = stomach). Gastrulation as it occurs in birds is not



GASTRULATION IN FORM WITH ISOLECITHAL EGG HAVING ALMOST NO YOLK—AMPHIOXUS.



GASTRULATION IN FORM WITH TELOLECITHAL EGG CONTAINING MODERATE AMOUNT OF YOLK—AMPHIBIA.



GASTRULATION IN FORM WITH TELOLECITHAL EGG CONTAINING LARGE AMOUNT OF YOLK—BIRDS.

FIG. 21.—Schematic diagrams to show the effect of yolk on gastrulation. Abbreviations: blc., blastocœle; bld., blastoderm; blp., blastopore; etc., ectoderm; ent., entoderm; mit., cell undergoing mitosis; yk., yolk; yk.g., yolk granules; yk.p., yolk plug.

difficult to understand if one grasps its fundamental similarity to the corresponding process in forms with less yolk. In Amphioxus, gastrulation is an inpocketing of the blastula

(Fig. 21). A double layered cup is formed from a single layered hollow sphere much as one might push in a hollow rubber ball with the thumb. The new cavity in the double walled cup is termed the gastrocœle or archenteron. The opening from the outside into the gastrocœle is called the blastopore. Thus in gastrulation the single cell layer of the blastula is doubled upon itself to form two layers. The outer cell layer is known as the ectoderm and the inner layer as the entoderm. These layers differ from each other in their positional relationship to the embryo and to the surrounding environment. Each has different functional potentialities and each will in the course of development give rise to quite different types of structures and organs. It is the importance of their later history rather than any complexity or veiled significance about the way in which they arise that attaches such importance in embryology to the establishment of these two layers.

In the gastrulation of Amphibian embryos (Fig. 21) the yolk forces the invagination of the blastoderm toward the animal pole, but the inpocketing takes place into the blastocœle and the interrelationships of ectoderm, entoderm, and gastrocœle are established in fundamentally the same way as in Amphioxus.

Gastrulation in birds is greatly modified by the large amount of yolk present (Fig. 21). Infolding must be effected in a disc of cells resting like a cap on a large yolk sphere. The smallness of the blastocœle sharply restricts the space in which the invagination[1] can occur. Instead of arising as a relatively large circular opening the blastopore appears as a crescentic slit at the margin of the blastoderm. The crescentic blastopore may be regarded as a potentially circular opening which has been flattened as it develops between the growing disc of cells and the unyielding yolk which underlies them. The invaginated pocket of entoderm which grows in this compressed blastopore is also flattened, conforming to the restrictions of the shape and size of the blastocœle. Moreover the floor of

[1] The term invagination is used in this book simply to mean infolding. It is not meant to imply (as embryologists sometimes use it in distinction to involution and epiboly) that the infolding in question occurred without differential growth as a primary causative factor. It seems to me of questionable wisdom to involve the beginner in a delicately shaded distinction between invagination, involution and epiboly based on the degree and direction of expression of differential growth.

the invagination is represented only by a few widely scattered cells lying upon the yolk. It is as if the lower layer in its ingrowth was impeded and broken up by the yolk. The scattered cells representing the floor of the invagination soon disappear and the yolk itself comes to constitute the floor of the gastrocœle. Notwithstanding the great displacement of the blastopore and the gastrular invagination toward the animal pole and the restricted size and incomplete floor of the gastrocœle, the cell layers and the cavity established can be homologized with the corresponding features in forms where the course of development has not been so extensively modified by yolk.

A comparative review of the diagrams of figure 21 will afford a general understanding of the infolding process of gastrulation. These diagrams aim to convey merely the scheme of the process. They are, therefore, simplified and emphasize the similarities of gastrulation in forms with widely varying amounts of yolk rather than the details of the process in any one form. With this general groundwork we may now profitably return to the blastula stage and consider in somewhat more detail the process of gastrulation as it occurs in birds.

Gastrulation in Birds.—We have already established the blastula as a disc of cells lying on the yolk but separated from it centrally by a flattened blastocœle or segmentation cavity. The peripheral part of the blastoderm where the marginal cells lie unseparated from the yolk has been termed the zone of junction (Fig. 22, *D*). This part of the blastoderm is also called the area opaca because in preparations made by removing the blastoderm from the yolk surface, yolk adheres to it and renders it more opaque. This opacity is especially apparent when a preparation is viewed under the microscope by transmitted light. The central area of the blastoderm, because it is separated from the yolk by the segmentation cavity, does not bring a mass of adherent yolk with it when the blastoderm is removed. It is for this reason translucent and is called the area pellucida. The area opaca later becomes differentiated so that three more or less distinct zones may be distinguished: (1) a peripheral zone, known as the margin of overgrowth, where rapid proliferation has pushed the cells out over the yolk without their becoming adherent to it; (2) an intermediate zone, known as the zone

of junction, in which the deep-lying cells do not have complete cell boundaries but constitute a syncytium blending without definite boundary into the superficial layer of white yolk and adhering to it by means of penetrating strands of cytoplasm; (3) an inner zone, known as the germ wall, made up of cells derived from the inner border of the zone of junction which have acquired definite boundaries and become more or less free from the yolk. The cells of the germ wall usually contain numerous small yolk granules which were enmeshed in their cytoplasm when they were, as cells of the zone of junction, unseparated from the yolk (Fig. 22, *B*, *E*). The inner margin of the germ wall marks the transition from area opaca to area pellucida.

The changes in the blastula which indicate the approach of gastrulation are, first, a thinning of the blastoderm at its caudal margin and, second, freeing of the blastoderm from the yolk in the same region (Fig. 22, *D*). The separation of the blastoderm from the yolk is evidenced in surface views by a crescentic gap in the posterior quadrant of the zone of junction (Fig. 22, *A*). This region where the blastoderm is thin and free from the yolk marks the position of the blastopore.

Gastrulation begins with the undertucking of the cells at the free margin of the blastoderm. Figure 22, *B*, is a diagrammatic surface view of the blastoderm represented as a transparent object. The position and the extent of the invaginated entoderm, seen through the overlying ectoderm, are indicated by the cross-hatched area. The appearance of the blastopore locates the caudal region of the future embryonic body and permits the definition of its longitudinal axis. This axis is indicated by the line *b–b* on figure 22, *B*. A diagram of a section cut in the longitudinal axis and passing through the blastopore of an embryo of this stage is shown in figure 22, *E*. The invaginated cells which constitute the entoderm form a layer extending cephalad from the thickened lip of the blastopore. The yolk forms the floor of the gastrocœle. Figure 22, *C*, is a diagrammatic surface view of a later stage in the same process. The extent of the entoderm is marked by cross-hatching as in the diagram of the previous stage. The undertucking of the cells at the blastopore has ceased by this time, and as indicated in figure 22, *C*, by the black area, and in figure

22, *F*, by the solid mass of cells seen in section, the blastopore has become closed.

During the entire time that the process of gastrulation is in progress there is constant cell proliferation going on in the

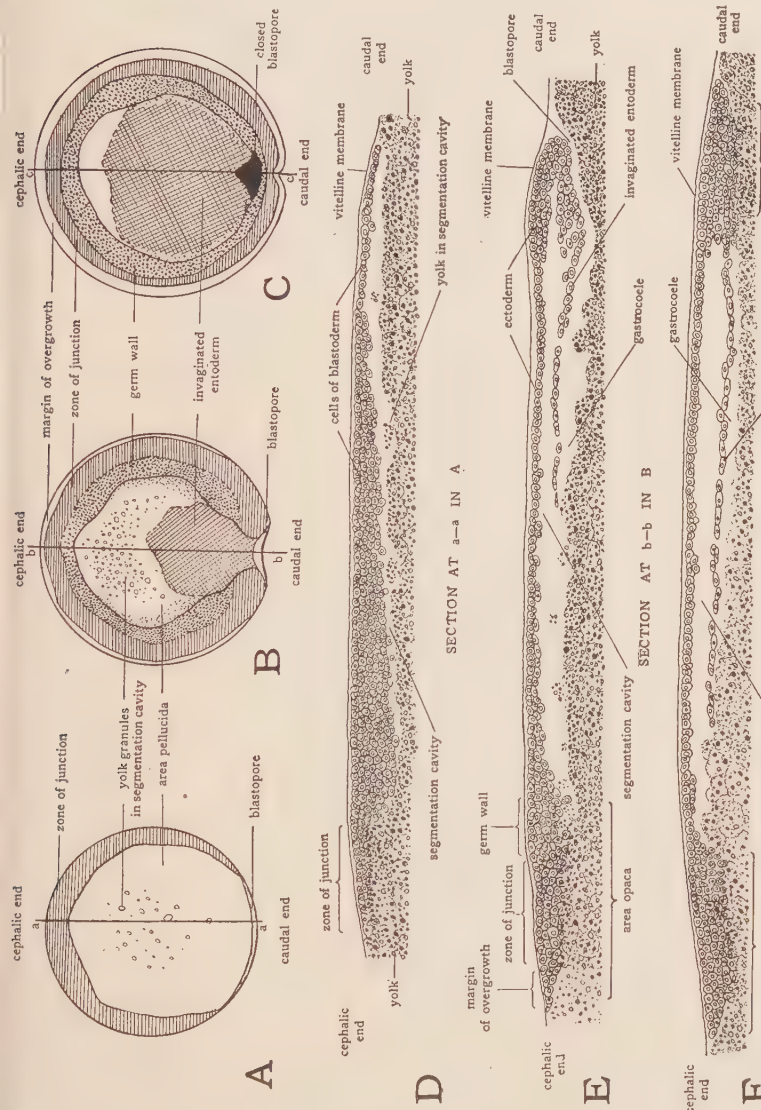


FIG. 22.—Diagrams to show various stages in the gastrulation of a bird embryo. (*After Patterson's figures for the pigeon.*) In the surface-view plans the blastoderm is supposed to be transparent so the underlying structures may be located. *A*, surface view of blastoderm just before invagination; *B*, surface view of blastoderm, invagination well advanced; *C*, surface view of blastoderm at end of gastrulation; *D*, vertical section through blastoderm of stage represented in *A*. The plane of the section is indicated by the line *a–a* in *A*. *E*, vertical section through blastoderm of stage represented in *B*. The plane of the section is indicated by the line *b–b* in *B*. *F*, vertical section through blastoderm of stage represented in *C*. The plane of the section is indicated by the line *c–c* in *C*.

blastoderm as a whole. The resultant growth of the blastoderm is evidenced by increase in its surface extent which entails a general spreading of its peripheral margins over the yolk.

This extension takes place uniformly at all parts of the margin except in the posterior quadrant where the blastopore is located. Here the cells proliferated, instead of spreading out over the yolk, turn in at the lip of the blastopore to form the entoderm. This particular part of the margin of the blastoderm, having contributed the cells formed in it to the entoderm which turns under the ectoderm and grows toward the center of the blastoderm, takes small part in the general peripheral expansion. As a result, the rapidly extending margins of the blastoderm on either side of the blastopore sweep around and enclose it. The blastopore at the time of its closure thus comes to lie within the recompleted circle of the germ wall (Fig. 22, *C*).

CHAPTER V

THE FORMATION OF THE PRIMITIVE STREAK AND THE ESTABLISHMENT OF THE MESODERM

THE LOCATION AND APPEARANCE OF THE PRIMITIVE STREAK; THE ORIGIN OF THE PRIMITIVE STREAK BY CONCRESCENCE OF THE MARGIN OF THE BLASTOPORE; THE FORMATION OF THE MESODERM; THE EMBRYOLOGICAL IMPORTANCE OF THE GERM LAYERS.

The Location and Appearance of the Primitive Streak.—The stages of development described in the preceding chapters take place before the egg is laid. The first conspicuous struc-

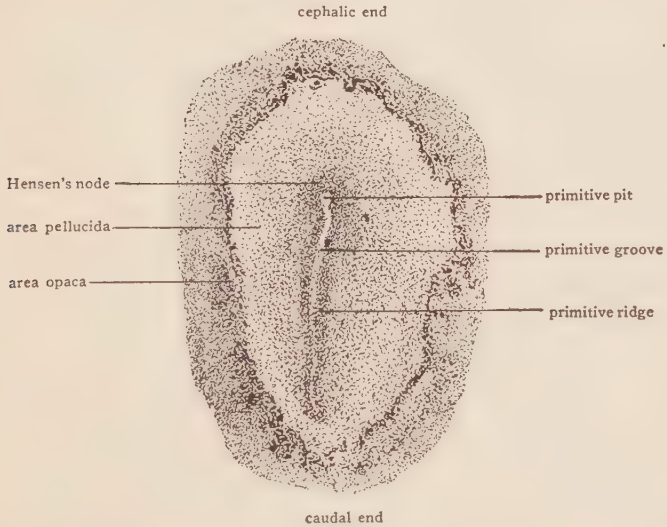


FIG. 23.—Dorsal view (× 14) of entire chick embryo in the primitive streak stage (about 16 hours of incubation).

tural feature to make its appearance in the embryo after the laying of the egg is the primitive streak. In eggs that have been incubated about 16 hours the primitive streak is well developed as a linear groove flanked on either side by ridge-like thickenings, extending from the inner margin of the area opaca to approxi-

mately the center of the blastoderm (Fig. 23). The primitive streak lies in the longitudinal axis of the future embryo. The end adjacent to the area opaca is its posterior (caudal) end, the opposite extremity is its anterior (cephalic) end. The cephalic end of the primitive groove is deepened and often somewhat expanded to form a depression known as the primitive pit. Directly anterior to the primitive pit the right and left primitive folds merge with each other in the mid-line to form a small rounded elevation called Hensen's node. Hensen's node is of importance as a landmark rather than because it gives rise to any particular structure.

As early as the beginning of gastrulation the shape of the blastoderm as a whole responds to local inequality in the rate of growth. One of the early manifestations of differential growth is the more rapid extension of the embryo cephalad than either laterad or caudad. By the time the primitive streak is well established differential growth has caused a marked elongation in the embryo's antero-posterior axis (Fig. 23).

The Origin of the Primitive Streak by Concrescence of the Margin of Blastopore.—The significance of the primitive streak has been the subject of much controversy. The divergences of opinion have been due chiefly to incomplete knowledge of the stages of development passed through prior to the laying of the egg. Our present knowledge of these early stages is, however, sufficient to furnish the basis of an interpretation of the primitive streak which is now widely accepted. This interpretation is outlined below without reference to other, opposed views.

The primitive streak is to be regarded as a scar-like thickening arising from the fusion of the edges of the anterior lip of the blastopore. To understand the origin of the longitudinally placed primitive streak from the marginally located crescentic blastopore it is necessary to follow carefully the growth processes taking place during the closure of the blastopore.

We have already seen how the ingrowth of entoderm from the anterior lip of the blastopore caused the blastopore to lag behind the other parts of the margin of the blastoderm in the process of radial extension over the yolk surface. During this process the blastopore is compressed from either side toward

the mid-line by the rapidly extending margins of the blastoderm adjacent to it and is eventually encompassed by them (see Chap. IV and Fig. 22). Because of the insweeping, converging tendency of the growth which first causes the blastopore to be laterally compressed and finally causes its margins to grow together, the process has been termed concrescence.

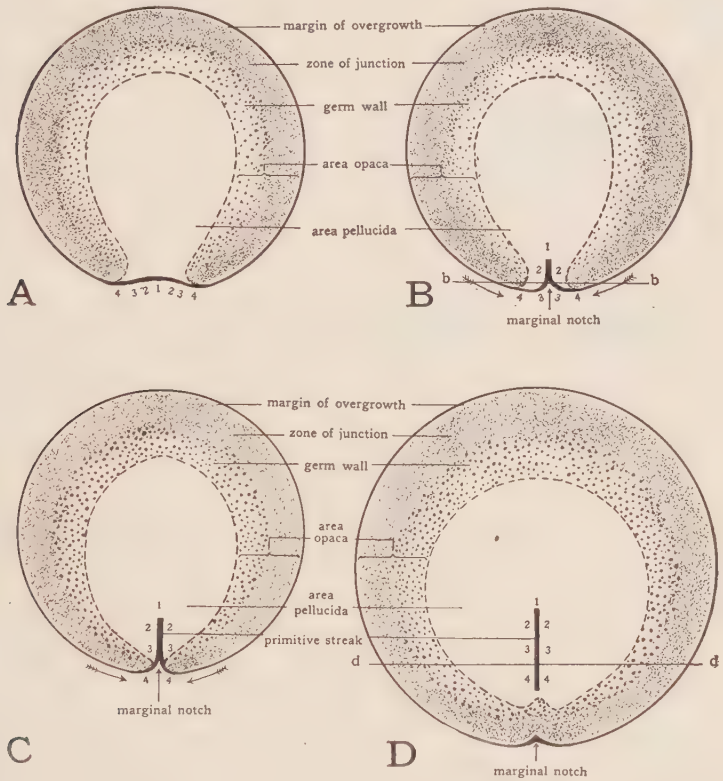


FIG. 24.—Schematic diagrams to illustrate the concrescence theory of the origin of the primitive streak. (*After Lillie.*) For explanation see text.

A schematic interpretation of four steps in the concrescence of the margins of the blastopore is given in the diagrams of figure 24. The blastoderm shown in surface-view plan in figure 24, *A*, is approximately at the same stage of gastrulation as that indicated in figure 22, *B*. To avoid complicating the diagram, the entoderm has not been shown in figure 24. Numbers have been placed along the lip of the blastopore to facilitate following the changes in position undergone by the points which they designate. As the margins of the blastoderm

adjacent to the blastopore grow, they tend to converge in the direction indicated by the arrows in figure 24, *B*. The anterior lip of the blastopore is folded on itself by this converging growth. The middle point of the lip, 1, comes to lie within the margin of the blastoderm, and points, 2, 2, which formerly lay laterally are brought into apposition in the mid line. Figures *C* and *D*, show how, by the continuation of the same converging growth, the edges of the blastopore are folded together into a line of fusion at right angles to the original marginal position of the blastopore. At the completion of concrescence, the germ wall of the blastoderm has coalesced posterior to the blastopore leaving the line along which the blastopore lips have fused within the area pellucida. The non-committal term primitive streak was given to this structure before its origin by fusion of the lips of the blastopore was suspected.

The Formation of the Mesoderm.—In its early condition the primitive streak is a scarcely recognizable thickening of the blastoderm marking the line of fusion of the lips of the blastopore. The well defined groove with thickened ridges on either side, seen in chicks of 15 to 16 hours incubation, is a later development. A new process, the formation of the mesoderm, is taking place at this region and the change in the configuration of the primitive streak is its outward manifestation. It will be recalled that the lip of the blastopore is in all forms a region of rapid cell proliferation. It is a region from which we can trace the addition of cells to the differentiated germ layers, but it is itself indifferent. Ectoderm and entoderm both merge into this indifferent area at the lip of the blastopore. It is impossible to fix, except arbitrarily, where ectoderm begins and entoderm ends. Later when the mesoderm appears, we can trace the origin of its cells directly or indirectly to the same area of indifferent, rapidly proliferating cells. It is therefore wholly in line with the embryology of other forms to find the mesoderm of the chick arising at the fused lips of the blastopore.

The manner in which the mesoderm arises can be understood only by the study of sections or diagrams of sections. Figure 25, *A*, represents schematically the conditions which would be seen in a section cut in the line *b–b* across the marginal notch of an embryo of the stage depicted in figure 24, *B*. The margins of the blastopore at the point where this section is located

have been folded so they lie in close proximity to each other. A little later they would be fused as shown in figure 25, *B*. At the region of fusion, that is to say at the primitive streak, the entoderm and ectoderm merge in a mass of rapidly dividing cells (Fig. 28, *D*). A section across the primitive streak at a somewhat later stage (Fig. 25, *C*) shows cells extending to either side of the undifferentiated cell mass, between the ecto-

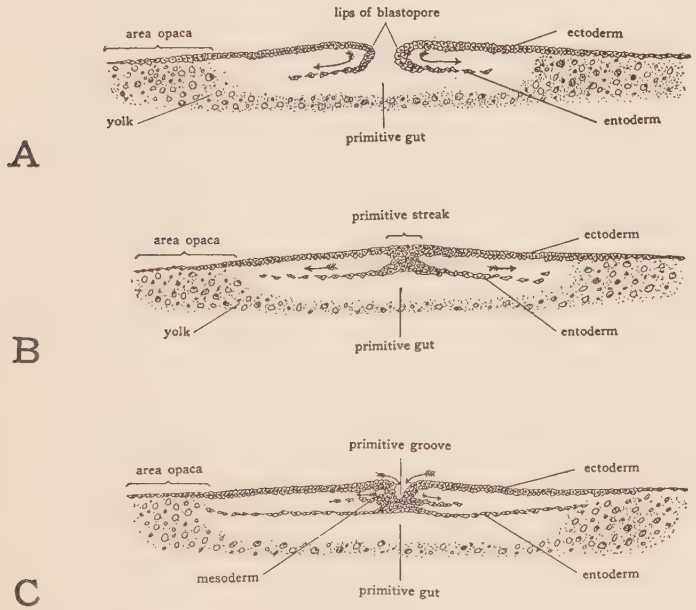


FIG. 25.—Diagrams showing schematically the relations of the germ layers during the formation of the primitive streak by concrescence of the margins of the blastopore. *A*, hypothetical section of blastoderm at the stage represented in Fig. 24, *B*. The plane of the section is indicated by the line *b–b* Fig. 24, *B*. *B*, hypothetical section of blastoderm at the stage represented in Fig. 24, *D*. The plane of the section is indicated by the line *d–d*, Fig. 24, *D*. *C*, schematic transverse section through the primitive streak at the stage represented in Fig. 23.

derm and the entoderm. These cells are the primordium of the third of the germ layers, the mesoderm. The outgrowth of the mesoderm and the median depression in the primitive streak appear synchronously. This median depression in the primitive streak is the primitive groove. It is not unlikely that the formation of the primitive groove is due to cell rearrangement in this region entailed by the rapid outgrowth of the cells constituting the mesoderm. (See arrows in figure 25, *C*.)

The Embryological Importance of the Germ Layers.—In looking back over the development thus far undergone by the embryo, perhaps the most conspicuous thing, at first glance, is the multitude of cells formed from the single fertilized egg cell. Of more significance, however, is the fact that even during the early phases of rapid proliferation the cells thus formed do not remain as an unorganized mass. Almost at once they become definitely arranged on the yolk sphere as the cap-like blastoderm. Scarcely is the blastoderm established when, at a definite marginal area, certain cells are undertucked and become arranged as a second layer beneath the first. This second layer because of its position inside the original layer is called the entoderm. Shortly a third cell-layer makes its appearance between the first two, being called, appropriately enough, the mesoderm. That part of the original cap of cells which still constitutes the outer layer after the entoderm and mesoderm have been established is now properly called the ectoderm. These three cell-layers are spoken of as the *germ layers*[1] of the embryo.

The germ layers are of interest to the embryologist from several points of view. The simple organization of the embryo when it consists of first a single, then two, and finally three primary structural layers is reminiscent of ancestral adult conditions that occur in primitive groups of the invertebrate series. From the standpoint of probable ontogenetic recapitulations of remote phylogenetic history several facts are quite suggestive. The nervous system of the vertebrate embryo arises from the ectoderm,—the layer through which a primitive organism which has not as yet evolved a central nervous system is in touch with its environment. The lining of the vertebrate digestive tube is formed from the entoderm,—the layer which in very primitive forms lines a gastrocœle-like enteric cavity. The vertebrate skeletal and circulatory structures are derived almost entirely from the mesoderm,—the layer which in small, lowly organized invertebrates is relatively inconspicuous, but which constitutes a progressively greater proportion of the

[1] British authors generally use instead of ectoderm, mesoderm, and entoderm,—epiblast, mesoblast and hypoblast. These terms have the disadvantage of not implying the layer-like character expressed by the root—**derm** (skin), but they have the advantage of emphasizing the actively growing character of the layers by the incorporation of the Greek root—**blast** meaning germ.

total bulk of animals as they increase in size and complexity and consequently need more elaborate supporting and transporting systems.

Interesting as are the possibilities of interpreting the germ layers from the standpoint of their phylogenetic significance, our chief concern with them centers about the part they play in the development of the individual. The establishment of the germ layers is the first segregation of cell groups which are clearly distinct from one another by reason of their definite relations within the embryo. The fact that these relations are fundamentally the same in all vertebrate embryos speaks forcefully of the common ancestry and similar heritage of the various members of this great group of animals. It means, furthermore, that in these germ layers we have a common starting point in the fabrication of the variations which different classes of animals have built upon the common underlying plan of body structure characteristic of the vertebrate group as a whole.

The establishment of the germ layers marks also a transition from the period of development when mere increase in number of cells is the outstanding event, to one in which differentiation and specialization are dominating concomitants of growth. Differentiation is occurring within the germ layers even before we can see tangible evidences of it by any of our present microscopical methods. Within a layer that looks all alike to us there are gradually being established localized groups of cells with different developmental potentialities. We have long known that such must be the case because we could see various special structures, one after another, take shape from a germ layer that gave no advance notice by any visible changes of what it was about to do. Recent experimental work, especially that of Hoadley (see Bibliography) is beginning to give us information as to how long such invisible differentiation precedes the visible morphological localization of a cell group which we readily recognize as the primordium of a definite organ. For example, although the optic vesicle as a definite primordium does not appear until about 30 hours of incubation, if a narrow transverse strip of the ectoderm of an 8 to 12-hour chick is cut out from the region either side of Hensen's node and grown outside the embryo, it will in a few days, show

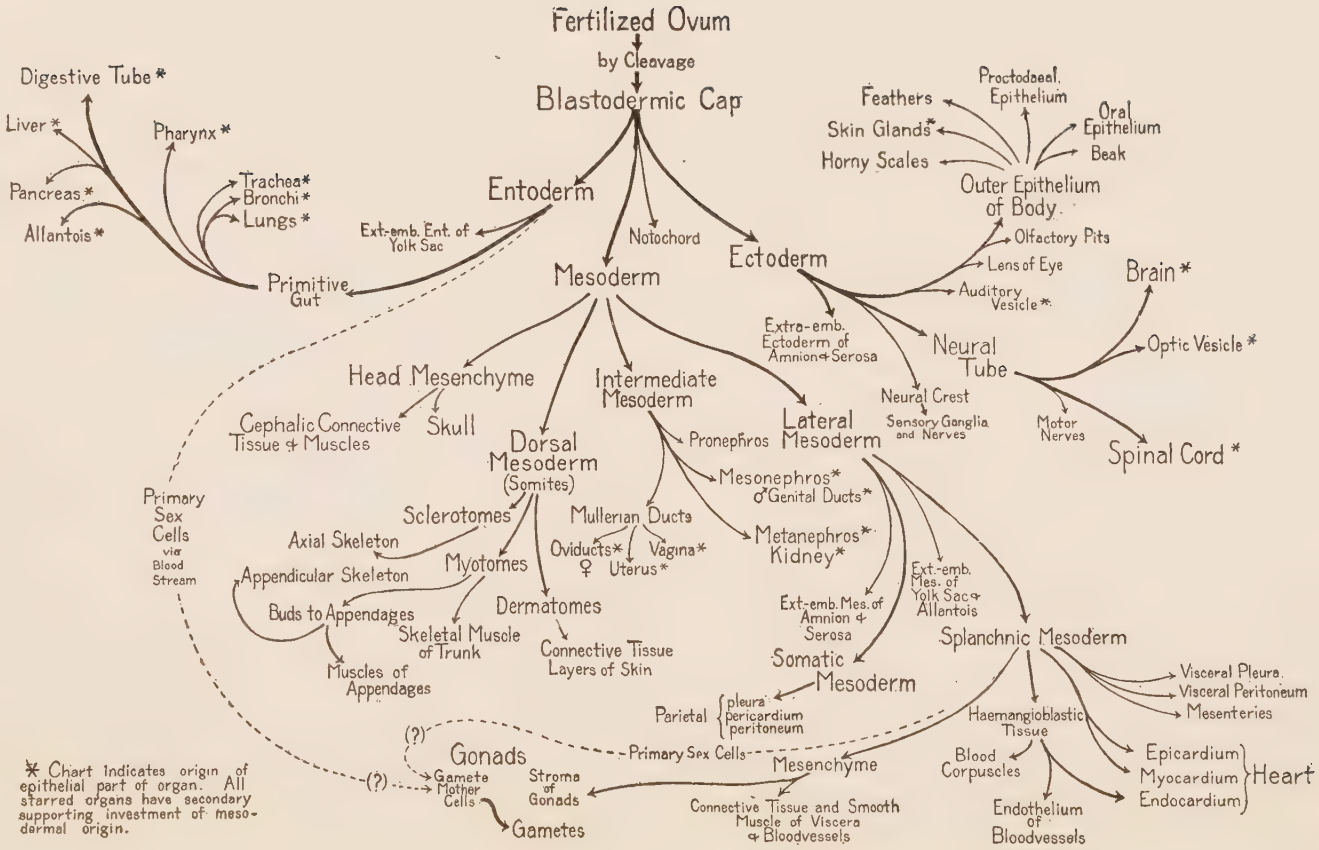


FIG. 26.—Chart showing the derivation of various parts of the body by progressive differentiation and divergent specialization. Note especially how the origin of all the organs can be traced back to the three primary germ layers.

specialized cellular elements of a type which occur only in the eye. A strip taken from another region, although it appears similar, will show no cells characteristic of the eye but will show different specific specializations. Experiments such as these indicate the surprisingly early stage at which there is, within the germ layers, invisible localization of cells with different developmental potentialities. As development progresses these localized cell groups are bodily and visibly sorted out. In some cases their sorting out is accomplished by a folding off from the parent germ layer, in other cases by migration of individual cells which later become re-aggregated elsewhere. From primordial cell groups thus derived the organs with which we are familiar in the adult gradually take shape. The story of the embryological origin of the various parts of the body is, therefore, the history of the growth, subdivision, and differentiation of the germ layers.

An outline plan of the repeated regroupings and progressive differentiations and specializations of the cells derived from the primary germ layers is given in figure 26. This chart at the present stage of our study will serve as a means of pointing out in a general way whither the early processes with which we have been dealing are destined to lead. As we follow the phenomena of development farther we shall find each natural division of the subject centers more or less sharply about some particular branch of this genealogical tree of the germ layers.

CHAPTER VI

FROM THE PRIMITIVE STREAK STAGE TO THE APPEARANCE OF THE SOMITES

THE PRIMITIVE STREAK AS A CENTER OF GROWTH; THE GROWTH OF THE ENTODERM AND THE ESTABLISHMENT OF THE PRIMITIVE GUT; THE GROWTH AND DIFFERENTIATION OF THE MESODERM; THE FORMATION OF THE NOTOCHORD; THE FORMATION OF THE NEURAL PLATE; THE DIFFERENTIATION OF THE EMBRYONAL AREA.

The Primitive Streak as a Center of Growth.—The importance of the primitive streak embryologically, is due chiefly to the way it is involved in the establishment of the germ layers and to its activity as a growth center. Representing as it does the fused lips of the blastopore it marks the location of entoderm invagination. The mesoderm also arises at the primitive streak. Subsequent to the establishment of these layers the region of the primitive streak continues to be especially active in growth, contributing to the expansion of all of the layers of the embryo. But to understand the importance of the primitive streak in this respect one must first become familiar with its location and structure.

The general appearance and the location of the primitive streak are both well shown in embryos of 16 hours of incubation (Fig. 23). In embryos which have been incubated 18 hours (Fig. 27) the primitive streak is still the most conspicuous structure. In form and size it is little changed from the conditions seen in 16 hour chicks, but it appears more caudally located. In 21 to 22-hour embryos (Fig. 31) the primitive streak lies still farther caudally in the blastoderm. This change in position, however, is relative rather than actual and is due to the fact that growth is especially rapid cephalic to the primitive streak.

The detailed structure of the primitive streak region is best shown by transverse sections. In the sections diagrammed in

figure 28, a different conventional scheme of representation has been employed to indicate each of the germ layers. The ectoderm is vertically hatched, the cells of the mesoderm are represented by heavy angular dots when they are isolated or by solid black lines when they lie arranged in the form of compact layers, and the entoderm is represented by fine stippling backed by a single line. A similar conventional representation of the different germ layers is observed in all diagrams of sections in order to facilitate following the way in which the organ systems of the embryo are constructed from the germ

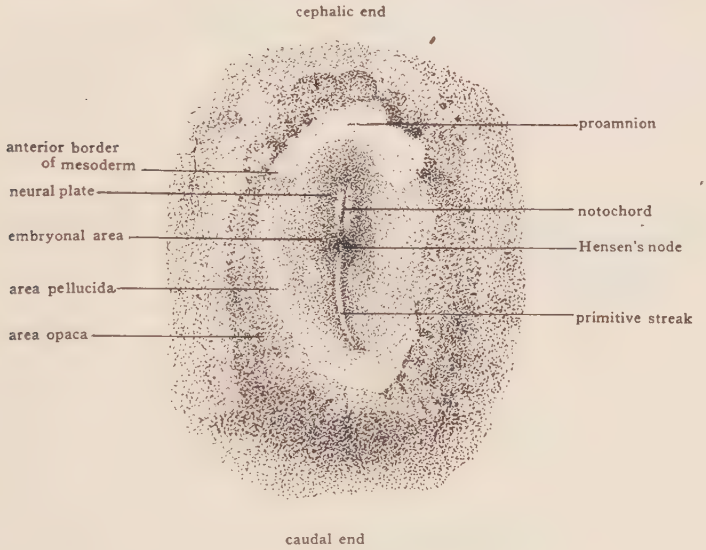


FIG. 27.—Dorsal view (× 14) of entire chick embryo of 18 hours incubation.

layers. Details of cell structure are for the most part omitted with the expectation that the student will acquire a knowledge of them in his own study of sections. The plane in which each of the sections diagrammed passes through the embryo is indicated by a line drawn on a small outline sketch of an embryo of corresponding stage. For interpretation these outline sketches should be compared with detailed drawings of entire embryos of the same stage of development, or better, with actual specimens.

In embryos of the stage under consideration the relationship of the germ layers at the primitive streak still indicates their manner of derivation (Fig. 28, *C* and *D*). The ectoderm and

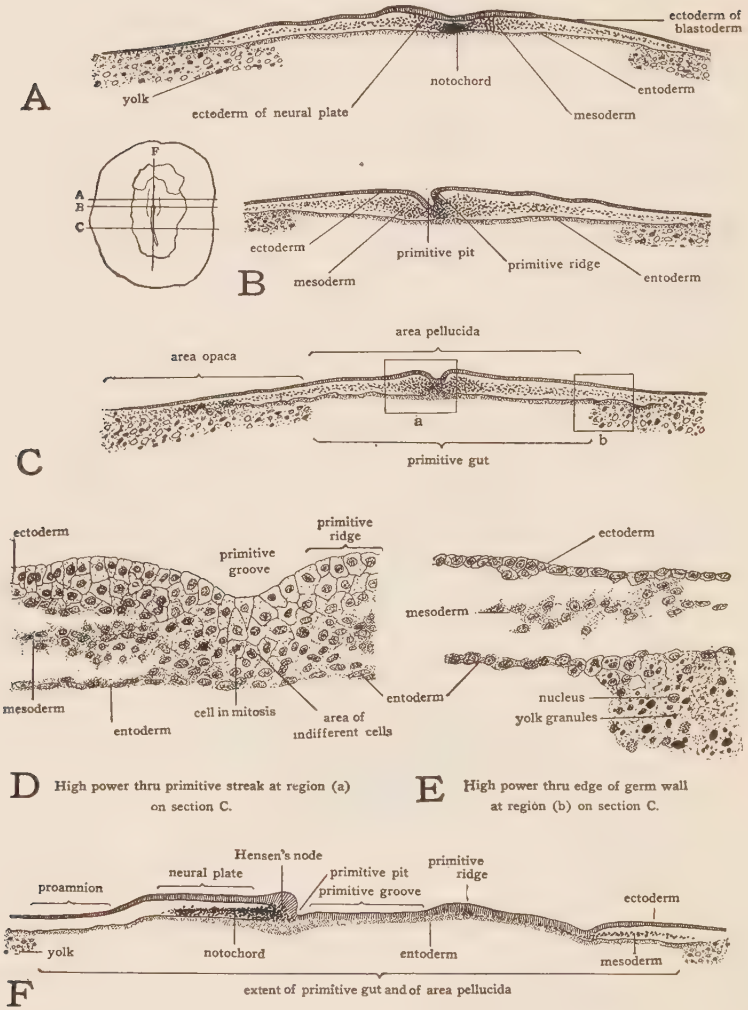


FIG. 28.—Sections of 18-hour chick. The location of each section is indicated by a line drawn on a small outline sketch of an entire embryo of corresponding age. The letters affixed to the lines indicating the location of the sections correspond with the letters designating the section diagrams. Each germ layer is represented by a different conventional scheme: ectoderm by vertical hatching; entoderm by fine stippling backed by a single line; and the cells of the mesoderm which at this stage do not form a coherent layer, by heavy angular dots.

A, diagram of transverse section through notochord; *B*, diagram of transverse section through primitive pit; *C*, diagram of transverse section through primitive streak; *D*, drawing showing cellular structure in primitive streak region; *E*, drawing showing cellular structure at inner margin of germ wall; *F*, diagram of median longitudinal section passing through notochord and primitive streak.

the entoderm are continuous with each other without any demarcation. The mesoderm arises from the primitive streak where ectoderm and entoderm merge. Growing laterad on either side of the primitive streak, it extends into the space between ectoderm and entoderm. The mass of cells in the floor of the primitive groove is to be regarded as constituting an undifferentiated area from which new cells are being proliferated rapidly and are emigrating to become components of one or another of the germ layers.

In figure 28, *D*, a small region at the primitive streak has been drawn at higher magnification to show the characteristic cellular structure of the undifferentiated region in the floor of the primitive groove and of the various layers merging at this place. The cells of the ectoderm are much more closely packed together and more sharply delimited than those of the other germ layers. Where the ectoderm is thickened in the primitive ridge region, it is several cell layers thick (stratified). (Fig. 28, *D*.) In regions lateral to the primitive ridge it gradually becomes thinner until it consists of but a single cell layer (Fig. 28, *E*). The rapid extension that the mesoderm is at this time undergoing is indicated by the loose arrangement and sprawling appearance of its cells. Their irregular cytoplasmic processes make them look much like amœbæ fixed during locomotion. The cells of the entoderm are neither as closely packed nor as clearly defined as are the ectoderm cells. Nevertheless, in contrast to the condition of the mesoderm at this stage, the entoderm cells form a definite, unbroken layer.

To those who have studied the embryology of more primitive vertebrates, particularly the Amphibia, the fact that the lips of the blastopore constitute centers of growth from which cells are pushed forth to take part in the formation of the differentiated germ layers will already be familiar. The fact that the blastopore of the chick has suffered a change in position due to concrescence, and has in the same process become closed by fusion of its lips to form the primitive streak must not be allowed to obscure its homologies. In attempting to bring the relationships of the germ layers in the chick into line with the relationships of the germ layers in embryos having less yolk, it will be of great assistance to picture a chick lifted off the yolk and the lateral margins of the blastoderm pulled

together ventrally (Fig. 29); or, the method of comparison may be reversed if one imagines the embryo of a form having less yolk, such as an amphibian, to be split open along the mid-ventral line and spread out on the surface of a sphere as a chick lies on the yolk.

Unless one realizes what happens to the cells formed in the primitive streak the importance of this region as a growth center is difficult to appreciate. For, in spite of the very

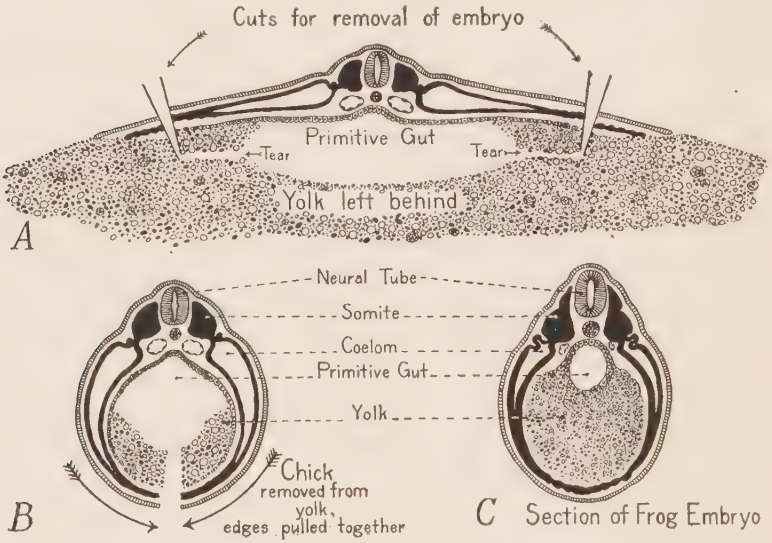


FIG. 29.—*A*, Diagram showing how the usual method of removing chick embryos from the yolk in order to prepare them for microscopical study makes the sections appear as if the primitive gut had no ventral boundary.

B and *C*, show how removing a chick from the yolk and pulling its edges together ventrally facilitates comparisons with forms which do not develop spread out on a large yolk sphere.

rapid cell division which occurs in it, the primitive streak does not increase in size. It becomes, on the contrary, a relatively less and less conspicuous structure and retains its original caudal position (see Figs. 23, 27, 32, and 36). The reason for this is the experimentally demonstrable fact that the cells proliferated in the primitive streak region do not remain there but push forth as fast as they are formed (Fig. 30, *D*, *E*). The great majority of the new cells are crowded in between the primitive streak and the already established part of the embryo cephalic to the streak. This results in rapid expansion of the

body anterior to the primitive streak. One is very likely, in observing a series of embryos in which the progress of elongation in the anterior region is so striking (Figs. 23, 27, 33, and 36), to attribute it entirely to especially active growth in this region itself. In reality it is due rather to rapid growth from behind, which pushes the cephalic region ahead of it.

The fact that the growth of a young embryo is taking place chiefly from its caudal end has a bearing also on the relative progress of differentiation in different regions of the body. It is a striking fact that the cephalic end of an embryo will always be found precocious in differentiation as compared with the more posterior portions of the embryo. This much-commented-on condition seems but natural when we consider that the head is actually older in development; for the structures posterior to the head are laid down by cells which were proliferated from the growth center at the primitive streak subsequently to the establishment of the head itself. Differentiation does occur exceedingly rapidly in the head. Were this not so, other regions would pass it in developmental progress. But we cannot, in taking cognizance of this condition, afford to overlook the fact that the head is given a considerable lead at the outset by its earlier establishment.

The Growth of the Entoderm and the Establishment of the Primitive Gut.—Sections of embryos of this stage show how the entoderm has spread out and become organized into a coherent layer of cells merging peripherally with the inner margin of the germ wall and overlapping it to a certain extent (Fig. 28, *C*, *E*, *F*). The cavity between the yolk and the entoderm which has been called the gastrocœle is now termed the primitive gut. The yolk floor of the primitive gut does not show in sections prepared by the usual methods. The reasons for this are to be found in the relations of the embryo to the yolk before it is removed for sectioning. In the entire central region of the blastoderm the yolk is separated from the entoderm by the cavity of the primitive gut. When the embryo is removed from the yolk sphere the yolk floor of the primitive gut, not being adherent to the blastoderm, is left behind (Fig. 29, *A*). In contrast, the peripheral part of the blastoderm lies closely applied to the yolk. Some yolk adheres to this part of the blastoderm when it is removed. This adherent yolk

is shown in the section diagrams of figure 28. Its presence clearly indicates why this region (area opaca) appears less translucent in surface views of entire embryos (Fig. 27).

In embryos of 18 hours the primitive gut is a cavity with a flat roof of entoderm and a floor of yolk. Peripherally it is bounded on all sides by the germ wall (Fig. 28, *C*, *F*). The merging of the cells of the entoderm with the yolk mass is shown in the small area of the germ wall drawn to a high magnification in figure 28, *E*. In the germ wall cell boundaries are incomplete and very difficult to distinguish, but nuclei can be made out surrounded by more or less definite areas of cytoplasm. This cytoplasm contains numerous yolk granules in various stages of absorption. It will be recalled that the nuclei of the germ wall arise by division from the nuclei of cells lying at the margins of the expanding blastoderm. They appear to be concerned in breaking up the yolk in advance of the entoderm as it is spreading about the yolk sphere.

About the twenty-second hour of incubation indications can be seen of a local differentiation of that region of the primitive gut which underlies the anterior part of the embryo. By focusing through the ectoderm in the anterior region of a whole-mount of this age a pocket of entoderm can be seen (Fig. 31). This entodermal pocket is the first part of the gut to acquire a floor, other than the yolk floor, and is called from its anterior position the fore-gut. Consideration of the fore-gut except to note the location of its first appearance can advantageously be deferred because its origin and relationships are more readily appreciated from the study of somewhat older embryos.

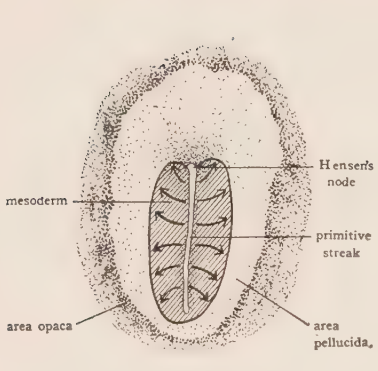
The Growth and Differentiation of the Mesoderm.—The mesoderm which arises from either side of the primitive streak spreads rapidly laterad and at the same time each lateral wing of the mesoderm swings cephalad. Figure 30 shows schematically the extension of the mesoderm during the latter part of the first day of incubation. The diagonal hatching represents the mesoderm seen through the transparent ectoderm. The principal landmarks of the embryos are sketchily represented.

It will be noticed that the manner in which the mesoderm spreads out leaves a mesoderm-free area in the anterior portion of the blastoderm. This region, known as the proamnion, is

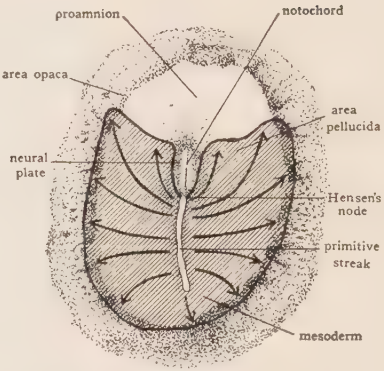
clearly recognizable in entire embryos by reason of its lesser density (Figs. 31 and 33). The name is unfortunate because of its false implication that this region is in some way a precursor of the amnion. Although it has long been known to be a misnomer the term proamnion is so deeply entrenched in the literature that it is difficult to dislodge. The only importance of this area is the information its rapidly diminishing size gives as to the rate of growth of the mesodermic wings which form its lateral borders (Fig. 30, *A-C*).

Sections passing through the primitive streak of embryos of this stage show loosely aggregated masses of mesoderm extending to either side between the ectoderm and entoderm. As would be expected from the method of origin, little mesoderm appears in the mid-line except posterior to the primitive streak. Immediately to either side of the mid-line the mesoderm is markedly thicker than it is farther laterad (Fig. 28, *A*). In whole-mounts the positions of the regional thickenings of the mesoderm are evidenced by the greater opacity they impart to the embryo locally (Fig. 31). These thickened zones of the mesoderm are the primordia of the dorsal mesodermic plates. Because of the way in which they are later divided into metamerically arranged cell masses or somites they are frequently designated as the segmental zones of the mesoderm. The segmental zones are in early stages most clearly marked somewhat cephalic to Hensen's node, where the first somites will appear. As they extend caudad on either side of the primitive streak they gradually become less and less definite.

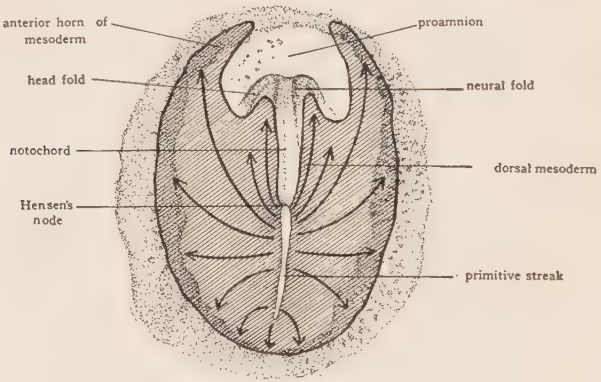
The sheet-like layers of mesoderm which are characteristic of the mid-body region do not extend to the anterior part of the embryo. The mesoderm of the head is derived from cells which become detached from the more definitely organized layers of mesoderm lying posteriorly, and migrate into the cephalic region. For this reason the cephalic mesoderm consists of loosely aggregated cells and never shows the regional differentiations and the organization into definite layers which later appear in the mesoderm of the mid-body region. To distinguish them from other parts of the mesoderm, aggregations of such wandering cells of mesodermal origin are called mesenchyme.



A CHICK OF ABOUT 14 HOURS.



B CHICK OF ABOUT 18 HOURS.



C CHICK OF ABOUT 22 HOURS.

D CHICK OF ABOUT 16 HOURS INJURED AT THE THREE POINTS MARKED.

E THE SAME CHICK AFTER FURTHER INCUBATION.

FIG. 30.—Diagrams showing direction of growth from the primitive streak as a center. *A* to *C*, show the growth as expressed by the progress of the mesoderm during the latter part of the first day of incubation. Some of the more prominent structural features of the embryos are drawn in lightly for orientation but the ectoderm is supposed to be nearly transparent allowing the mesoderm to show

The Formation of the Notochord.—The notochord arises in the chick as a median outgrowth from the rapidly proliferating, undifferentiated cells at the cephalic end of the primitive streak (Fig. 28, *F*). The way in which the notochord grows cephalad from the anterior end of the primitive streak, just as in other vertebrate embryos it arises from the region of the anterior lip of the blastopore, is one of the points which confirms the identification of the primitive streak of the chick as the closed blastopore.

Largely because of the way in which the notochord arises in Amphioxus, a primitive vertebrate of doubtful relationships, it has usually been considered of entodermal origin. In Amphibia and in birds it arises not from any definite germ layer but from the undifferentiated growth center about the blastopore which is giving rise to both entoderm and mesoderm. Even in Amphioxus the notochord arises at the same time and in the same manner as the mesoderm. In its later differentiation the notochord resembles mesodermal derivatives more closely than entodermal. The common origin of notochord and mesoderm, and the unmistakably mesodermal characteristics of the fully developed notochord should be emphasized rather than the early association of the notochordal primordium with the entoderm and its doubtful origin therefrom. For these reasons the notochord is in this book treated as a mesodermal structure.

In entire embryos of 18 to 22 hours (Figs. 27 and 31) the notochord can be seen in the mid-line extending cephalad from Hensen's node. Hensen's node is at once the posterior limit of the notochord and the anterior end of the primitive streak. The notochord and the primitive streak together clearly mark the mid-line of the embryo and establish definitely the longitudinal axis of the developing body. In sections (Fig. 28, *A*, *F*) the notochord is not at this early stage sharply differentiated from the loosely arranged mesoderm cells adjacent to it. In later stages, however, the cells composing it become aggregated to form a characteristic rod-shaped structure, circular in cross section and with clearly defined boundaries (Figs. 34 and 84, *C*).

through. The areas into which the mesoderm has grown are indicated by diagonal hatching.

D and *E* show the direction of growth as demonstrated by experimental methods. (*After Kopsch.*) *D*, shows the location at which three injuries were made close to the primitive streak of a 16-hour embryo. *E* shows the position to which the injured areas were carried by growth of the same embryo subsequent to the operation.

The Formation of the Neural Plate.—In surface views of entire chicks of about 18 hours (Fig. 27) areas of greater density may be made out on either side of the notochord. These areas extend somewhat anterior to the cephalic end of the notochord where they appear to blend with each other in the mid-line. Sections of this region (Fig. 28, *A*) show that the greater density seen in whole-mounts is due to thickening of the ecto-

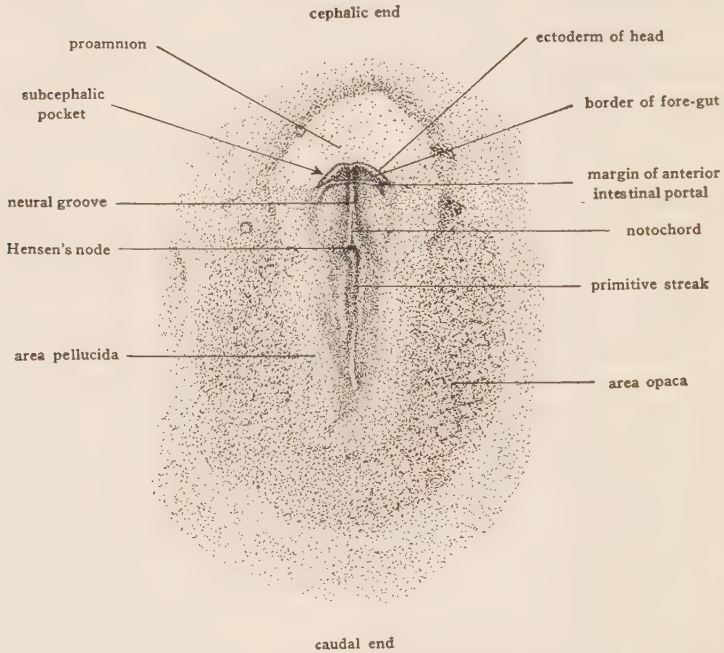


FIG. 31.—Dorsal view (× 14) of entire chick embryo of about 21 hours incubation.

derm. Rapid cell proliferation has resulted in the ectoderm in the middle region becoming several cells in thickness. This thickened area is known as the neural (medullary) plate. Laterally the thickened ectoderm of the neural plate blends without abrupt transition into the thinner ectoderm of the general blastodermic surface. Anteriorly the neural plate is more clearly marked than it is posteriorly. At the level of Hensen's node the neural plate diverges into two elongated areas of thickening, one on either side of the primitive streak.

In embryos of 21 or 22 hours (Fig. 31) the neural plate becomes longitudinally folded to establish a trough known as

the neural groove. The bottom of the neural groove lies in the mid-dorsal line. Flanking the neural groove on each side is a longitudinal ridge-like elevation involving the lateral portion o the neural plate. These two elevations which bound the neural groove laterally are known as the neural folds. The folding of the originally flat neural plate to form a gutter, flanked on either side by parallel ridges, is an expression of the same extremely rapid cell proliferation which first manifested itself in the local thickening of the ectoderm to form the neural plate. The formation of the neural plate and its subsequent folding to form the neural groove are the first indications of the differentiation of the central nervous system.

The Differentiation of the Embryonal Area.—Due to the thickening of the ectoderm to form the neural plate and also to the thickening of the dorsal zones of the mesoderm, the part of the blastoderm immediately surrounding the primitive streak and notochord has become noticeably more dense than that in the peripheral portion of the area pellucida. Because it is the region in which the embryo itself is developed this denser region is known as the embryonal area. Although the embryonal area is at this early stage directly continuous with the peripheral part of the blastoderm without any definite line of demarcation, they later become folded off from each other. The peripheral portion of the blastoderm is then spoken of as extra-embryonic because it gives rise to structures which are not built into the body of the embryo, although they play a vital part in its nutrition and protection during development.

The anterior region of the embryonal area is thickened and protrudes above the general surface of the surrounding blastoderm as a rounded elevation. This prominence marks the region in which the head of the embryo will develop (Fig. 31). The crescentic fold which bounds it is termed the head fold and is the first definite boundary of the body of the embryo. Throughout the course of development we shall find the head region farther advanced in differentiation than other parts of the body. The importance of differential growth as a factor in establishing this condition ontogenetically has already been discussed. Those familiar with comparative anatomy will see in this precocity of the head a repetition of race history in the development of the individual, for phylogenetically the head is

the oldest and most highly differentiated region of the body. This cephalic precocity of the embryo is but one of many manifestations of the law of recapitulation, in conformity with which the individual in its development rapidly repeats the main steps in the development of the race to which it belongs.

CHAPTER VII

THE STRUCTURE OF TWENTY-FOUR HOUR CHICKS

The formation of the head; the formation of the neural groove; the regional divisions of the mesoderm; the cœlom; the pericardial region; the area vasculosa.

The Formation of the Head.—In embryos of 21 to 22 hours the anterior part of the embryonal area is thickened and elevated above the level of the surrounding blastoderm, with a well defined crescentic fold marking its anterior boundary. Between 21 and 24 hours this region has undergone rapid growth (Fig. 32). Its elevation above the blastoderm is much more marked and it has grown anteriorly so it overhangs the proamnion region. The crescentic fold which formerly marked its anterior boundary appears to have undercut the anterior part of the embryo and separated it from the blastoderm. The changes in relationships are due, however, not so much to a posterior movement of the fold as to the anterior growth of the embryo itself. This anterior region which projects, free from the blastoderm, may now properly be termed the head of the embryo. The space formed between the head and the blastoderm is called the subcephalic pocket (Fig. 35, *E*).

In the mid-line the notochord can be seen through the overlying ectoderm. It is larger posteriorly near its point of origin than it is anteriorly. Nevertheless it can readily be traced into the cephalic region where it will be seen to terminate somewhat short of the anterior end of the head (Fig. 33).

The Formation of the Neural Groove.—The neural plate in chicks of 18 hours was seen as a flat, thickened area of the ectoderm. In embryos of 21 to 22 hours a longitudinal folding had involved it, establishing the neural groove in the mid-dorsal line flanked on either side by the neural folds. At 24 hours of incubation the folding of the neural plate is much more clearly marked. In transparent preparations of the entire embryo (Fig. 33) the neural folds appear as a pair of dark bands. The

folding which establishes the neural groove takes place first in the cephalic region of the embryo. At its cephalic end the neural groove is, therefore, deeper and the neural folds are correspondingly more prominent than they are caudally. The folding has not, at this stage, been carried much beyond the cephalic half of the embryo. Consequently as the neural folds are followed caudad they diverge slightly from each other, and become less and less distinct.

FIG. 32.—Chick embryo of 25–26 hours photographed by reflected light to show its external configuration. Compare with figures 33 and 34.

Study of transverse sections of an embryo of this stage affords a clearer interpretation of the conditions in neural groove formation than the study of entire embryos. A section passing through the head region (Figs. 3 and 35, *A*) shows the neural plate folded so it forms a nearly complete tube. Dorsally the margins of the neural folds of either side have approached each other and lie almost in contact. The formation of the neural folds takes place first in about the center of the head region, and progresses thence cephalad and caudad. By following caudad the sections of a transverse series, the margins of the

neural folds will be seen less and less closely approximated to each other.

The Establishment of the Fore-gut.—In the outgrowth of the head, the entoderm as well as the ectoderm has been involved. As a result the entoderm forms a pocket within the ectoderm, much like a small glove finger within a larger. This entodermic

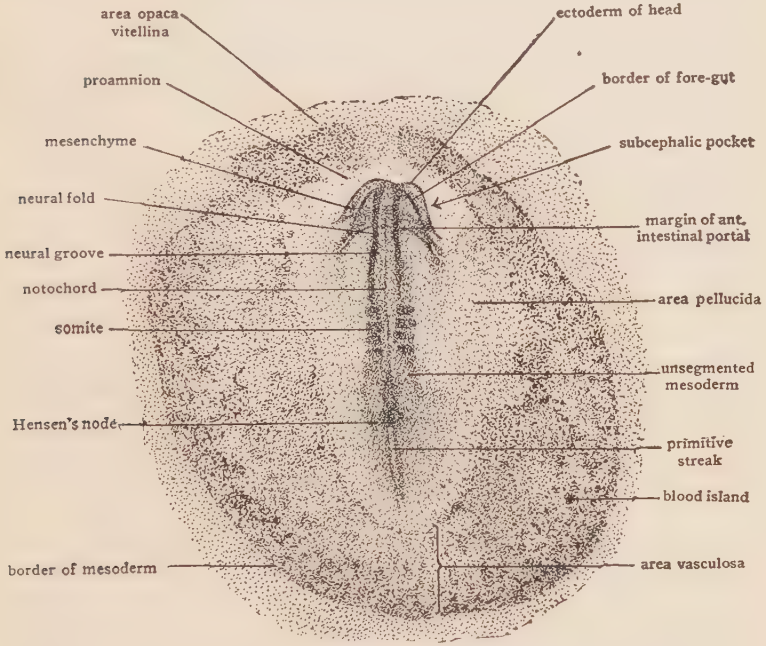


FIG. 33.—Dorsal view (× 14) of entire chick embryo having 4 pairs of mesodermic somites (about 24 hours incubation). Compare this figure of an embryo which has been stained and cleared and then drawn by transmitted light, with the preceding figure which shows an embryo of about the same age photographed by reflected light.

pocket, or fore-gut, is the first part of the digestive tract to acquire a definite cellular floor. That part of the gut caudal to the fore-gut, where the yolk still constitutes the only floor, is termed the mid-gut. The opening from the mid-gut into the fore-gut is called the anterior intestinal portal (fovea cardiaca).

The topography of the fore-gut region at this stage can be made out very well by studying the ventral aspect of entire embryos. The margin of the anterior intestinal portal appears as a well defined crescentic line (Fig. 34). The lateral boundaries of the fore-gut can be seen to join the caudally directed

tips of the crescentic margin of the portal. Considerably cephalic to the intestinal portal an irregularly recurved line can be made out. On either side it appears to merge with the ectoderm of the head. This line marks the extent to which the head is free from the blastoderm. It is due to the fold at the bottom of the subcephalic pocket where the ectoderm of the under surface of the head is continuous with the ectoderm of the blastoderm. Comparison of figure 34 with the sagittal section diagrammed in figure 35, *E*, will aid in making clear the relationships of fore-gut to the head. From the sagittal section

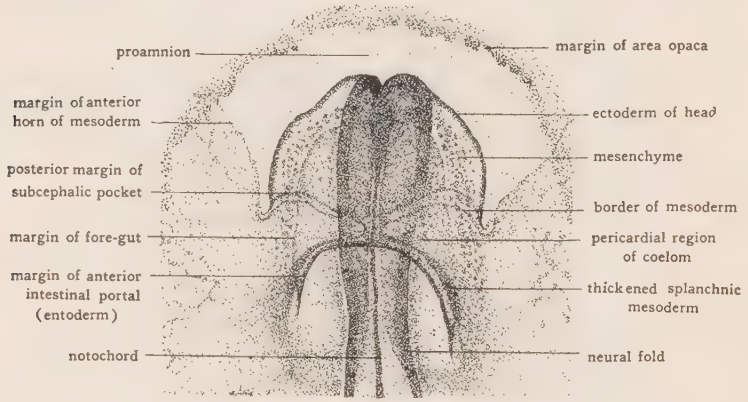


FIG. 34.—Ventral view (× 37) of cephalic region of chick embryo having 5 pairs of somites (about 25–26 hours of incubation).

it will also be apparent why the margins of the intestinal portal and of the subcephalic pocket appear as dark lines in the whole-mount. In viewing an entire embryo under the microscope by transmitted light one depends largely on differences in density for locating deep-lying structures. When a layer is folded so the light must pass through it edgewise, the fold stands out as a dark line by reason of the greater thickness it presents (Figs. 2 and 3).

The Regional Divisions of the Mesoderm.—The first conspicuous metamerically arranged structures to appear in the chick are the mesodermic somites. The somites arise by division of the mesoderm of the dorsal or segmental zone to form block-like cell masses. In the embryo shown in figure 33 three pairs of somites are completely delimited and a fourth pair can be made out which is not as yet completely cut off from the dorsal mesoderm posterior to it.

The regular addition of somites as embryos increase in age makes the number of somites the most reliable criterion of the stage of development. Chicks which have been incubated for a given number of hours show wide variation in the degree of development attained; chicks of a given number of somites vary but little among themselves.

Cross sections passing through the mid-body region show the formation of the somites and the beginning of other changes in the mesoderm (Fig. 35, *C*, cf. also Fig. 46, *E*). Following the mesoderm from the mid-line toward either side three regions or zones can be made out: (1) the dorsal mesoderm which at this level has been organized into somites, (2) the intermediate mesoderm, a thin and narrow plate of cells connecting the dorsal and lateral mesoderm, and (3) the lateral mesoderm which is distinguished from the intermediate by being split into two layers with a space between them.

The somites are compact cell masses lying immediately lateral to the neural folds. The cells composing them have a fairly definite radial arrangement about a central cavity which is very minute or wanting altogether when the somites are first formed but which later becomes enlarged (Fig. 62). Cephalic and caudal to the region in which somites have been formed the dorsal mesoderm is differentiated from the rest of the mesoderm simply by its greater thickness and compactness.

In 24-hour embryos the intermediate mesoderm shows very little differentiation. In the chick it never becomes segmentally divided as does the dorsal mesoderm. The fact that it is potentially segmental in character is indicated, however, by the way in which it later gives rise to segmentally arranged nephric tubules. Because of the part it plays in the establishment of the excretory system the intermediate mesoderm is frequently called the nephrotomic plate.

In the chick the lateral mesoderm, like the intermediate mesoderm, shows no segmental division. In 24-hour embryos (Fig. 35, *C*) it is clearly differentiated from the intermediate mesoderm by being split horizontally into two layers with a space between them. The layer of lateral mesoderm lying next to the ectoderm is termed the somatic mesoderm, the layer next to the entoderm is termed the splanchnic mesoderm, and the cavity between somatic and splanchnic mesoderm is the

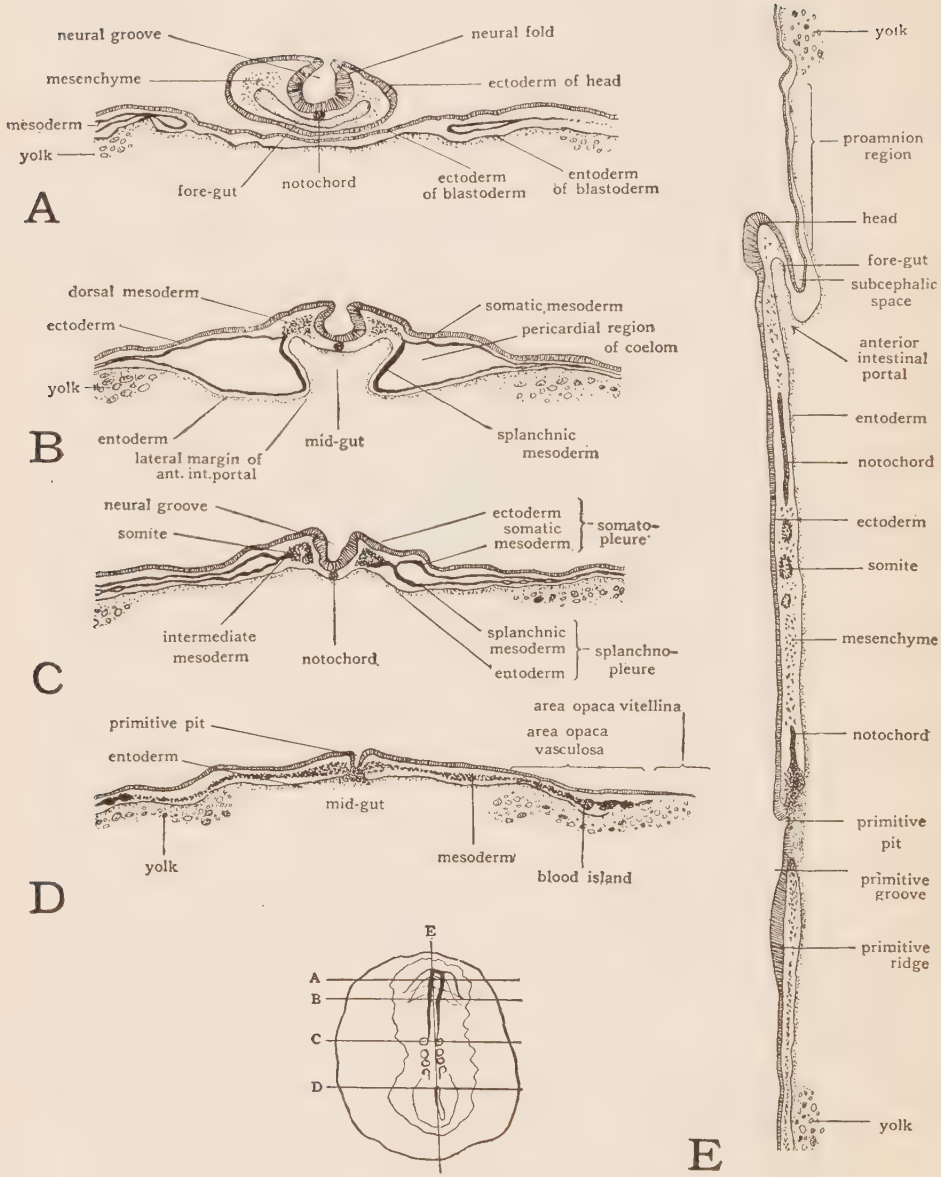


FIG. 35.—Diagrams of sections of 24-hour chick. The sections are located on an outline sketch of the entire embryo. The conventional representation of the germ layers is the same as that employed in Fig. 28 except that here where its cells have become aggregated to form definite layers the mesoderm is represented by heavy solid black lines.

cœlom. Because in development the somatic mesoderm and ectoderm are closely associated and undergo many foldings in common, it is convenient to designate the two layers together by the single term somatopleure. Similarly the splanchnic mesoderm and the entoderm together are designated as the splanchnopleure.

The Cœlom.—The cœlom, like the cell layers of the blastoderm, extends over the yolk peripherally beyond the embryonal area (Fig. 35, *C*). Later in development foldings mark off the embryonic from the extra-embryonic portion of the germ layers. This same folding process divides the cœlom into intra-embryonic and extra-embryonic regions. In the 24-hour chick, however, embryonic and extra-embryonic cœlom have not been separated.

It is evident from the manner in which the cœlomic chambers arise in the lateral mesoderm that the cœlom of the embryo consists of a pair of bilaterally symmetrical chambers. It is not until later in development that the right and left cœlomic chambers become confluent ventrally to form an unpaired body cavity such as is found in adult vertebrates.

The Pericardial Region.—In the region of the anterior intestinal portal the cœlomic chambers on either side show very marked local enlargement. Later in development these dilated regions are extended mesiad and break through into each other ventral to the fore-gut to form the pericardial cavity. In their early condition these enlarged regions of the cœlomic chambers are usually called amnio-cardiac vesicles. With their later fate in mind we may avoid multiplication of terms and speak of them from their first appearance as constituting the pericardial region of the cœlom.

The relationships of the pericardial region of the cœlom in embryos of 24 hours can most readily be grasped from a study of transverse sections. Figure 35, *B*, shows the great dilation of the cœlom on either side of the anterior intestinal portal as compared with its condition farther caudad (Fig. 35, *C*). Where the splanchnic mesoderm lies closely applied to the entoderm at the lateral margins of the portal it is noticeably thickened. It is from these areas of thickened splanchnic mesoderm that the paired primordia of the heart will later arise.

In entire embryos of this age the thickened splanchnic mesoderm can be made out as a dark band lying close against the crescentic entodermal border of the anterior intestinal portal (Fig. 34). If the preparation is favorably stained the boundaries of the pericardial regions of the cœlom can be traced (see Fig. 34). Following mesiad from the easily located thickened areas, the mesodermic borders can be seen to extend from either side, parallel to the entodermic margins of the portal, nearly to the mid-line. They then turn cephalad. When they encounter the ectodermal fold which constitutes the posterior boundary of the subcephalic pocket they swing laterad parallel with it and can be traced outside the embryonic region where they constitute the cephalic borders of the anterior horns of the mesoderm (see also Fig. 45, *A*).

The portion of the cœlom, the borders of which we have just located between the subcephalic pocket and the anterior intestinal portal, is an important landmark from another standpoint than the part it is destined to play in the formation of the pericardial region. It is the most cephalic part of the cœlom. There is no cœlom in the head. In the head region the mesoderm is not aggregated into definite masses or coherent cell layers. The mesodermic structures of the head are derived from cells which migrate into the cephalic region from the mesoderm lying farther caudally. It will be recalled that these migrating cells of mesodermal origin are termed mesenchymal cells, or collectively mesenchyme, to distinguish them from the more definitely aggregated cell layers of the mesoderm. It should be born in mind that the term is a general one, and later in development we shall encounter mesenchymal aggregations in many other regions of the body. By careful focusing on the whole-mount the mesenchyme of the head can be seen as an indefinite mass lying between the superficial ectoderm and the entoderm of the fore-gut. The distribution of the mesenchymal cells and the characteristic irregularity of shape correlated with their active amœboid movement may readily be made out from sections (Fig. 35, *A*).

The Area Vasculosa.—In a 24-hour chick the boundary between the area opaca and area pellucida has the same appearance and significance as in chicks of 18 to 20 hours. There is, however, a very marked difference between the proximal portion

of the area opaca adjacent to the area pellucida and the more distal portions of the area opaca. The proximal region is much darker and has a somewhat mottled appearance (Fig. 33). The greater density of this region is due to its invasion by mesoderm which makes it thicker and therefore more opaque in transmitted light (Fig. 35, *D*). The boundary between the inner and outer zones of the area opaca is established by the extent to which the mesoderm has grown peripherally. The distal zone is called the area opaca vitellina because the yolk alone underlies it. The proximal zone into which mesoderm has grown is known as the area opaca vasculosa, because it is from the mesoderm in this region that the yolk-sac blood vessels arise. The mottled appearance of this region is due to the aggregation of mesoderm into cell clusters, or blood islands, which mark the initial step in the formation of blood vessels and blood corpuscles. Later in development the formation of blood islands and vessels extends in toward the body of the embryo from its place of earliest appearance in the area opaca and involves the mesoderm of the area pellucida. The histological nature of the blood islands will be taken up in connection with later stages where their development is more advanced.

CHAPTER VIII

THE CHANGES BETWEEN TWENTY-FOUR AND THIRTY-THREE HOURS OF INCUBATION

THE CLOSURE OF THE NEURAL GROOVE; THE DIFFERENTIATION OF THE BRAIN REGION; THE ANTERIOR NEUROPORE; THE SINUS RHOMBOIDALIS; THE FATE OF THE PRIMITIVE STREAK; THE LENGTHENING OF THE FORE-GUT; THE APPEARANCE OF THE HEART AND OMPHALOMESENTERIC VEINS; ORGANIZATION IN THE AREA VASCULOSA.

In dealing with developmental processes the selection of stages for detailed consideration is more or less arbitrary and largely determined by the phenomena one seeks to emphasize. There is no stage of development which does not show something of interest. It is impossible in brief compass to take up at length more than a few stages. Nevertheless it is important not to lose the continuity of the processes involved. By calling attention to some of the more important intervening changes, this brief chapter aims to bridge the gap between the 24-hour stage and the 33-hour stages of the chick both of which are taken up in some detail.

The Closure of the Neural Groove.—In comparison with 24-hour chicks, entire embryos of 27 to 28 hours of incubation (Fig. 36) show marked advances in the development of the cephalic region. The head has elongated rapidly and now projects free from the blastoderm for a considerable distance, with a corresponding increase in the depth of the subcephalic pocket and in the length of the fore-gut.

In 24-hour chicks the anterior part of the neural plate is already folded to form the neural groove. Although the neural folds are at that stage beginning to converge mid-dorsally the groove nevertheless remains open throughout its length (Fig. 35, *A*, *B*, *C*). By 27 hours the neural folds in the cephalic region meet in the mid-dorsal line and their edges become fused.

The fusion which occurs is really a double one. Careful following of figures 44, *A* to *E*, will aid greatly in understanding the process. Each neural fold consists of a mesial component which is thickened neural plate ectoderm, and a lateral component which is unmodified superficial ectoderm (Fig. 44, *A*). When the neural folds meet in the mid-dorsal line (Fig. 44, *B*, *C*) the mesial, neural plate components of the two folds fuse with

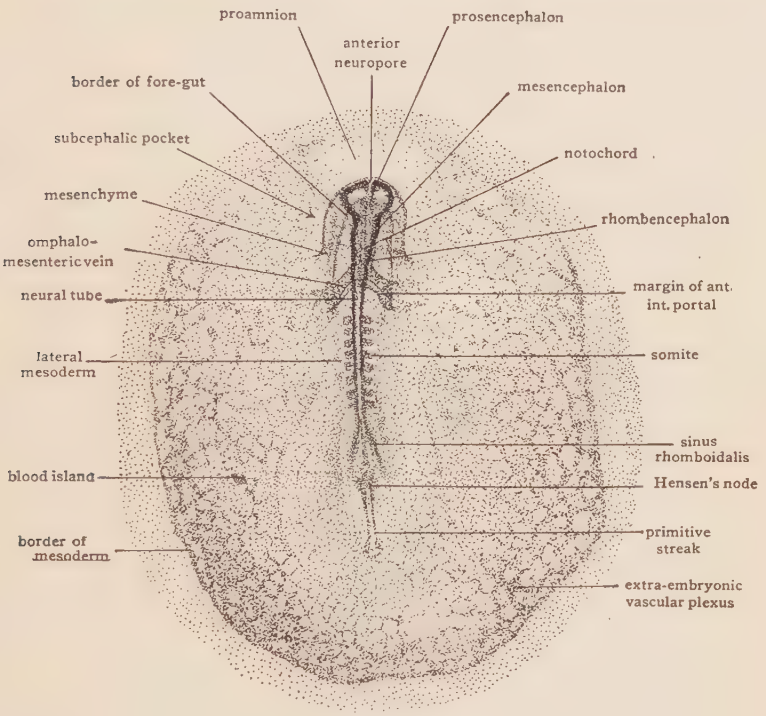


FIG. 36.—Dorsal view (× 14) of entire chick embryo having 8 pairs of somites (about 27–28 hours incubation).

each other, and the outer layers of unmodified ectoderm also become fused (Fig. 44, *D*). Thus in the same process the neural groove becomes closed to form the neural tube and the superficial ectoderm closes over the place formerly occupied by the open neural groove. Shortly after this double fusion the neural tube and the superficial ectoderm become somewhat separated from each other leaving no hint of their former continuity (Fig. 44, *E*).

The Differentiation of the Brain Region.—By 27 hours of incubation the anterior part of the neural tube is markedly enlarged as compared with the posterior part. Its thickened walls and dilated lumen mark the region which will develop into the brain. The undilated posterior part of the neural tube gives rise to the spinal cord. Three divisions, the three primary brain vesicles, can be distinguished in the enlarged cephalic

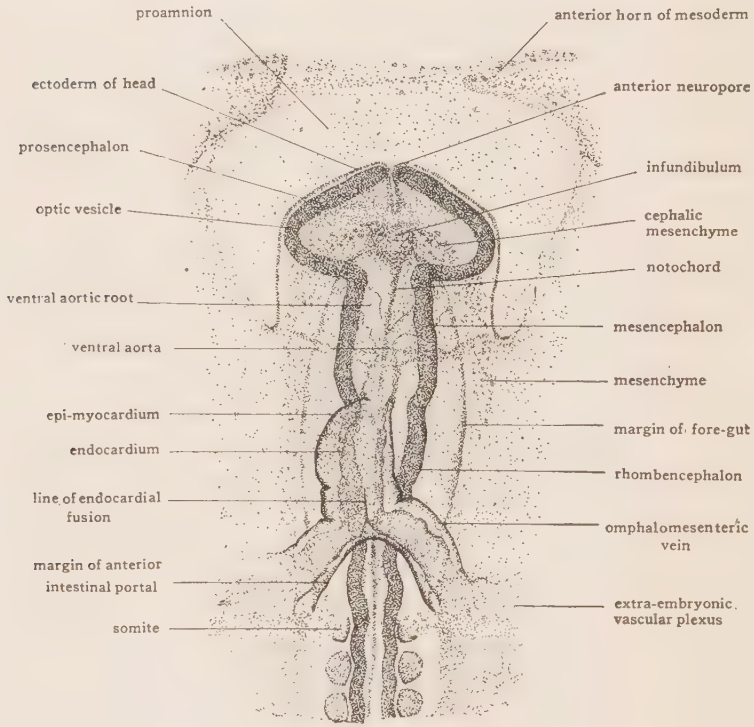


FIG. 37.—Ventral view (× 45) of head and heart region of chick embryo of 9 somites (about 29–30 hours incubation).

region of the neural tube (Fig. 36). Occupying most of the anterior part of the head is a conspicuous dilation known from its position as the fore-brain or prosencephalon. Posterior to the prosencephalon and marked off from it by a constriction is the mid-brain or mesencephalon. Posterior to the mesencephalon with only a very slight constriction marking the boundary is the hind-brain or rhombencephalon. The rhombencephalon is continuous posteriorly with the cord region of the neural tube without any definite point of transition.

In somewhat older embryos (Fig. 37) the lateral walls of the prosencephalon become out-pocketed to form a pair of rounded dilations known as the primary optic vesicles. When the optic vesicles are first formed there is no constriction between them and the lateral walls of the prosencephalon, and the lumen of each optic vesicle communicates mesially with the lumen of the prosencephalon without any definite line of demarcation.

The relation of the notochord to the divisions of the brain is of importance in later developmental processes. The notochord extends anteriorly as far as a depression in the floor of the prosencephalon known as the infundibulum (Fig. 37). Therefore, the rhombencephalon, mesencephalon, and that part of the prosencephalon posterior to the infundibulum lie immediately dorsal to the notochord (are epichordal) while the infundibular region and the parts of the prosencephalon cephalic to it project anterior to the notochord (are prechordal).

The Anterior Neuropore.—The closure of the neural folds takes place first near the anterior end of the neural groove and progresses thence both cephalad and caudad. At the extreme anterior end of the brain region closure is delayed. As a result the prosencephalon remains for some time in communication with the outside through an opening called the anterior neuropore. The anterior neuropore is still open in chicks of 27 hours (Fig. 36). In embryos of 33 hours the neuropore appears much narrowed (Fig. 39). A little later it becomes closed but leaves for some time a scar-like fissure in the anterior wall of the prosencephalon (Fig. 41). The anterior neuropore does not give rise to any definite brain structure. It is important simply as a landmark in brain topography. Long after it has disappeared as a definite opening, the scar left by its closure serves to mark the point originally most anterior in the developing brain.

The Sinus Rhomboidalis.—The rhombencephalic region of the brain merges caudally without any definite line of demarcation into the region of the neural tube destined to become the spinal cord. The neural tube as far caudally as somite formation has progressed is completely closed and of nearly uniform diameter. Caudal to the most posterior somites the neural groove is still open and the neural folds diverge to either side of Hensen's node (Fig. 36). In their later growth caudad the

neural folds converge toward the mid-line and form the lateral boundaries of an unclosed region at the posterior extremity of the neural tube known because of its shape as the sinus rhomboidalis (Fig. 39). Hensen's node and the primitive pit lie in the floor of this as yet unclosed region of the neural groove and subsequently are enclosed within it when the neural folds here finally fuse to complete the neural tube.

This process in the chick is homologous with the enclosure of the blastopore by the neural folds in lower vertebrates. In forms where the blastopore does not become closed until after it is surrounded by the neural folds, it for a time constitutes an opening from the neural canal into the primitive gut known as the neurenteric canal or posterior neuropore. In the chick the early closure of the blastopore precludes the establishment of an open neurenteric canal but the primitive pit represents its homologue.

The Fate of the Primitive Streak.—In embryos of about 27 hours the primitive streak is relatively much shorter than in younger embryos (Cf. Figs. 23, 27, 31, 33, and 36). This is due partly to its being overshadowed by the rapid growth of structures lying cephalic to it, and partly to actual decrease in the length of the primitive streak itself. The locally accelerated growth, which in very young embryos was so pronounced in the region of the primitive streak, slows up after the body as a whole has been established. The less active cells which have not emigrated thence, gradually lose their characteristic arrangement and this early landmark becomes less and less conspicuous as development advances. By the time the caudal end of the body is delimited, the primitive streak as a definitely organized structure has disappeared altogether (Cf. Figs. 36, 39, 47 and 55).

The Formation of Additional Somites.—The division of the dorsal mesoderm to form somites begins to be apparent in embryos of about 22 hours. By the end of the first day three or four pairs of somites have been cut off (Fig. 33). As development progresses new somites are added caudal to those first formed. In embryos which have been incubated about 27 hours eight pairs of somites have been established (Fig. 36).

It was formerly believed that some new somites were formed anterior to the first pair. The experiments of Patterson would

seem to indicate quite definitely that the first pair of completely formed somites remains the most anterior and that all the new somites are added posterior to them. The experiments referred to were carried out on eggs which had been incubated up to the time of the formation of the first somite. With thorough aseptic precautions the eggs were opened and the first somite marked, in some cases by injury with an "electric needle" in other cases by the insertion of a minute glass pin. Following the operation the shell was closed by sealing over the opening a piece of egg shell of appropriate size. After being again incubated for varying lengths of time the eggs were reopened. In all cases the injured first somite was still the most anterior complete somite. All the new somites except the incomplete "head somite" had appeared caudal to the first pair of somites formed.

The Lengthening of the Fore-gut.—Comparison of therelations of the crescentic margin of the anterior intestinal portal in embryos between 24 and 30 hours shows it occupying progressively more caudal positions (Fig. 45). This change in the position of the anterior intestinal portal is the result of two distinct growth processes. The margins of either side of the portal are constantly converging toward the mid-line where they become fused with each other. Their fusion lengthens the fore-gut by adding to its floor and thereby displacing the crescentic margin of the portal caudad. At the same time the structures cephalic to the anterior intestinal portal are elongating rapidly so that the portal becomes more and more remote from the anterior end of the embryo. This results in further lengthening of the fore-gut.

As a result of these two processes the space between the subcephalic pocket and the margin of the anterior intestinal portal is also elongated (Fig. 45). This is of importance in connection with the formation of the heart; for it is into this enlarging space that the pericardial portions of the cœlom extend, and within it that the heart comes to lie.

The Appearance of the Heart and Omphalomesenteric Veins.—Although the early steps in the formation of the heart take place in embryos of this range, detailed consideration of them has been deferred to be taken up in connection with later

stages when conditions in the circulatory system as a whole are more advanced.

In dorsal views of entire embryos the heart is largely concealed by the overlying rhombencephalon (Fig. 36) but it may readily be made out by viewing the embryo from the ventral surface (Fig. 37). At this stage the heart is a nearly straight tubular structure lying in the mid-line ventral to the fore-gut. Its mid-region has noticeably thickened walls and is somewhat dilated. Anteriorly the heart is continuous with a large median vessel, the ventral aorta, posteriorly it is continuous with the paired omphalomesenteric veins. The fork formed by the union of the omphalomesenteric veins in the posterior part of the heart lies immediately cephalic to the crescentic margin of the anterior intestinal portal, the veins lying within the fold of entoderm which constitutes its margin.

Organization in the Area Vasculosa.—The extra-embryonic vascular area at this stage is undergoing rapid enlargement and presents a netted appearance instead of being mottled as in the earlier embryos. The peripheral boundary of the area vasculosa is definitely marked by a dark band, the precursor of the sinus terminalis (marginal sinus). Its netted appearance is due to the extension and anastomosing of blood islands. The formation of the network is a step in the organization of a plexus of blood vessels on the yolk surface which will later be the means of absorbing and transferring food material to the embryo. The afferent yolk-sac or vitelline circulation is established in the next few hours of incubation when this plexus of vessels developing on the yolk surface comes into communication with the omphalomesenteric veins already developing within the embryo and extending laterad. The efferent vitelline circulation is established somewhat later when the omphalomesenteric arteries arise from the aorta of the embryo and become connected with the yolk-sac plexus. (Cf. Figs. 36, 39 and 47.)

CHAPTER IX

THE STRUCTURE OF CHICKS BETWEEN THIRTY-THREE AND THIRTY-NINE HOURS OF INCUBATION

THE DIVISIONS OF THE BRAIN AND THEIR NEUROMERIC STRUCTURE; THE AUDITORY PITS; THE FORMATION OF EXTRA-EMBRYONIC BLOOD VESSELS; THE FORMATION OF THE HEART; THE FORMATION OF INTRA-EMBRYONIC BLOOD VESSELS.

Chicks which have been incubated from 33 to 39 hours are in a favorable stage to show some of the fundamental steps in the formation of the central nervous system, and of the circulatory system. In this chapter, therefore, attention has been concentrated on these two systems.

During this period of incubation there are also changes in the fore-gut region and in the somites, and differentiation in the intermediate mesodern which presages the formation of the urinary organs. Consideration of these structures has, however, been deferred until their development has progressed somewhat farther.

The Divisions of the Brain and Their Neuromeric Structure. The metameric arrangement of structures which is so striking a feature in the body organization of all vertebrates, is masked in the head region of the adult by superimposed specializations. In the brain of young vertebrate embryos, however, the metamerism is still indicated. Dissections of the neural plate of chicks at the end of the first day of incubation show a series of eleven enlargements marked off from each other by constrictions (Fig. 38, *A*). Concerning the precise homologies of individual enlargements with specific neuromeres in other forms there is not complete agreement. The controversies center about the question of neuromeric fusions in the anterior part of the brain. For the beginning student the fact that metamerism is present is to be emphasized rather than the controversies concerning the homologies of neuromeres. With the

reservation that some of the anterior enlargements may represent fusions of more than one neuromere, the series of enlarge-

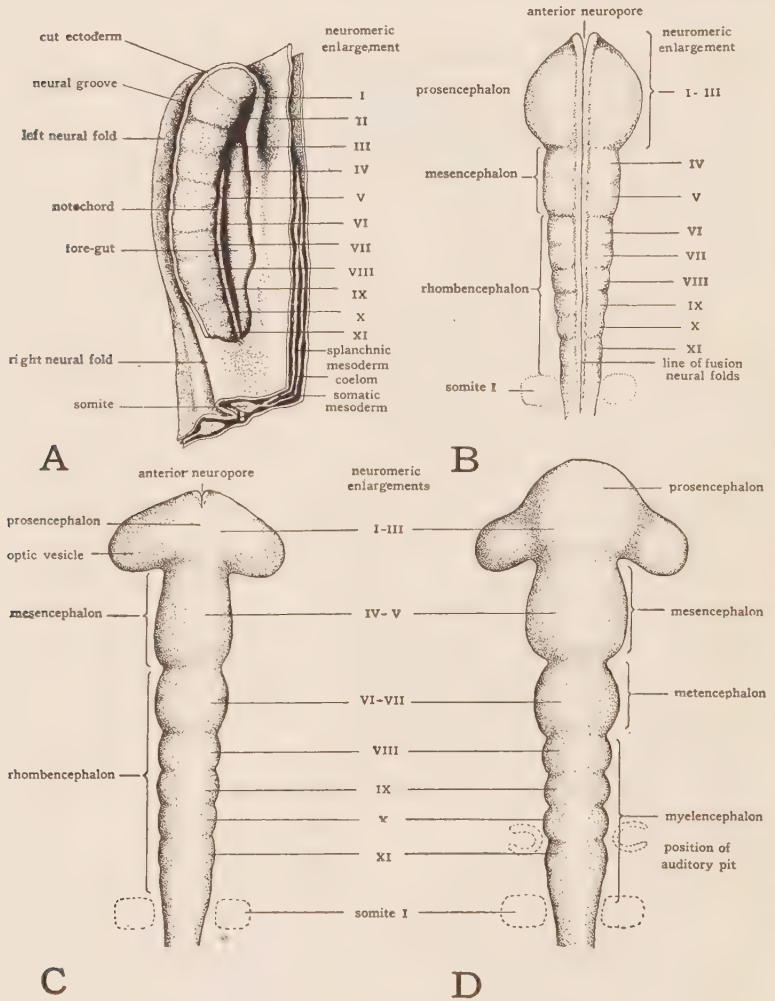


FIG. 38.—Diagrams to show the neuromeric enlargements in the brain region of the neural tube. (*Based on figures by Hill.*)

A, lateral view of neural plate from dissection of chick of 4 somites (24 hours); *B*, dorsal view of brain dissected out of 7-somite (26 to 27-hour) embryo; *C*, dorsal view of brain from 10-somite (30-hour) embryo; *D*, dorsal view of brain from 14-somite (36-hour) embryo.

ments seen in the brain region of the chick may be regarded as neuromeric. For convenience in designation the neuromeres are numbered beginning at the anterior end.

With the closure of the neural tube and the establishment of the three primary brain vesicles we can begin to trace the fate of the various neuromeric enlargements in the formation of the

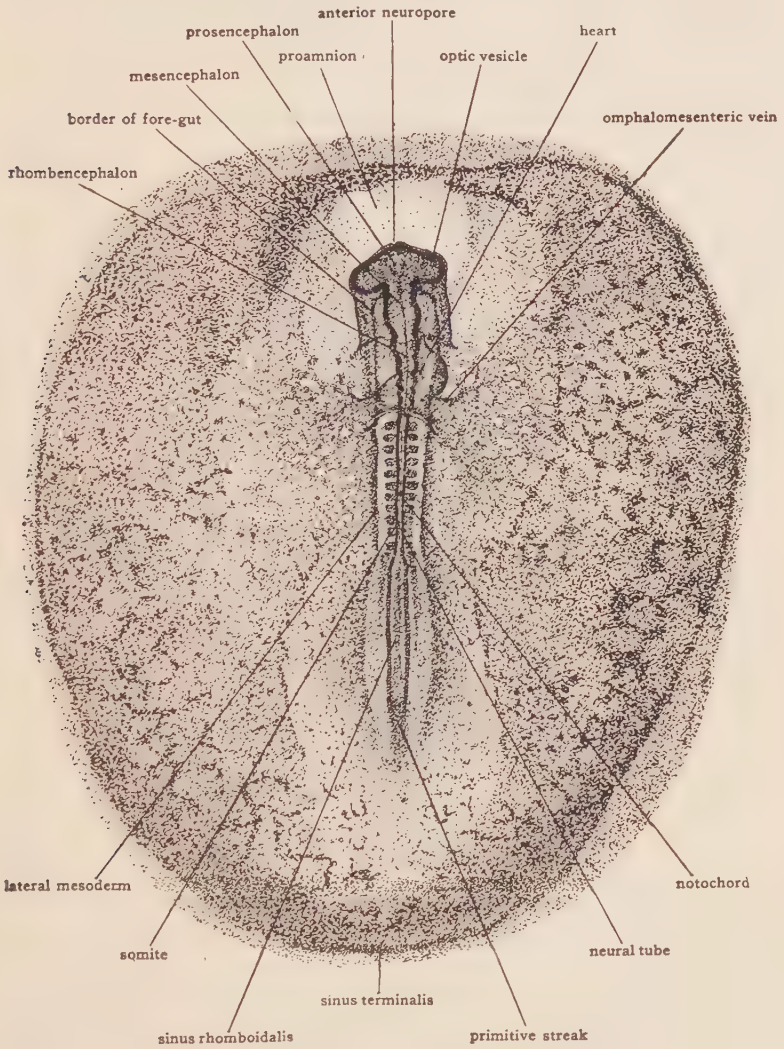


FIG. 39.—Dorsal view (× 14) of an entire chick embryo of 12 somites (about 33 hours incubation).

brain regions. The three anterior neuromeres form the prosencephalon; neuromeres four and five are incorporated in the mesencephalon; and neuromeres six to eleven in the rhombencephalon (Fig. 38, *B*). Anteriorly the interneuromeric

constrictions soon disappear except for two; namely, the one between the prosencephalon and mesencephalon, and the one between the mesencephalon and rhombencephalon. The rhombencephalic neuromeres, however, remain clearly marked for a considerable period.

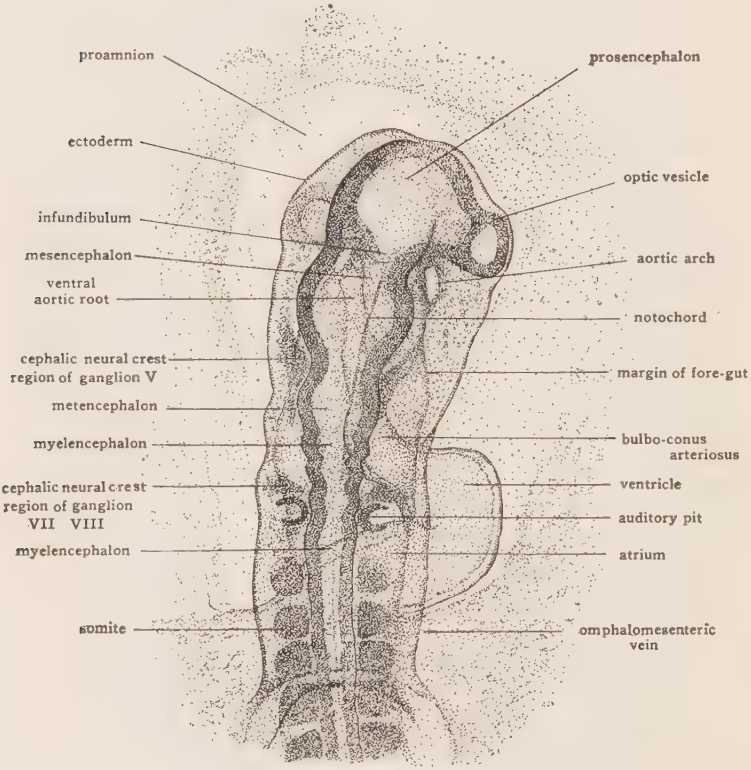


FIG. 40.—Dorsal view (× 45) of head and heart region of a chick embryo of 17 somites (38–39 hours incubation).

By about 33 hours of incubation the optic vesicles are established as paired lateral outgrowths of the prosencephalon. They soon extend to occupy the full width of the head (Fig. 38, *C* and Fig. 39). The distal portion of each of the vesicles thus comes to lie closely approximated to the superficial ectoderm, a relationship of importance in their later development. At first the cavities of the optic vesicles (opticœles) are broadly confluent with the cavity of the prosencephalon (prosocœle). Somewhat later constrictions appear which mark more defi-

nitely the boundaries between the optic vesicles and the prosencephalon (Fig. 38, *D* and Fig. 40).

Concurrently there has been formed in the floor of the prosencephalon a depression, known because of its peculiar shape as the infundibulum (Figs. 41 and 42). The infundibular

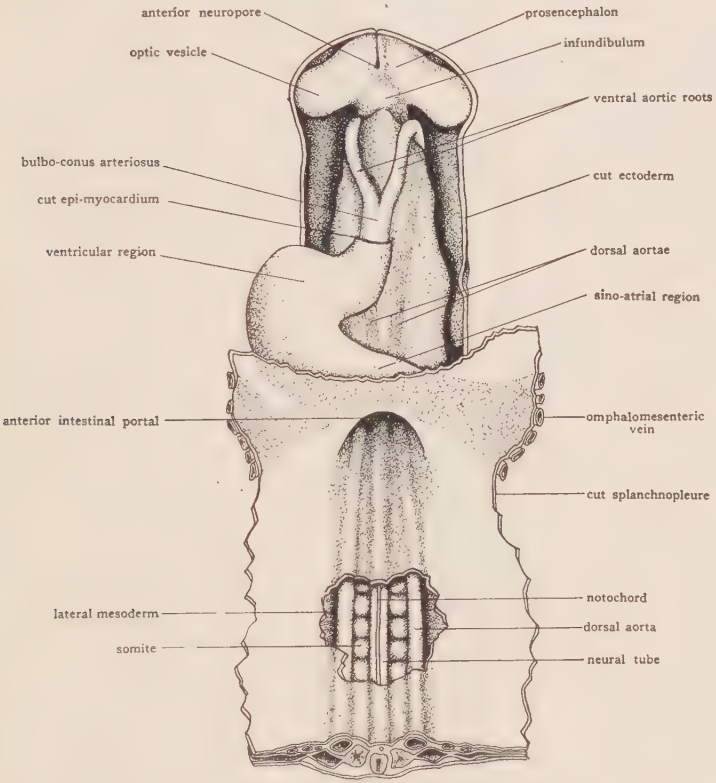


FIG. 41.—Diagrammatic ventral view of dissection of a 35-hour chick embryo. (*Modified from Prentiss.*) The splanchnopleure of the yolk-sac cephalic to the anterior intestinal portal, the ectoderm of the ventral surface of the head, and the mesoderm of the pericardial region, have been removed to show the underlying structures. Figure 42 should be referred to for the relations of the pericardial mesoderm.

region is the site of important changes later in development. At this stage, conditions are not sufficiently advanced to warrant more than calling attention to its origin from the prosencephalon, and its positional relation to the anterior end of the notochord.

In chicks of about 38 hours indications of the impending division of the three primary vesicles to form the five regions

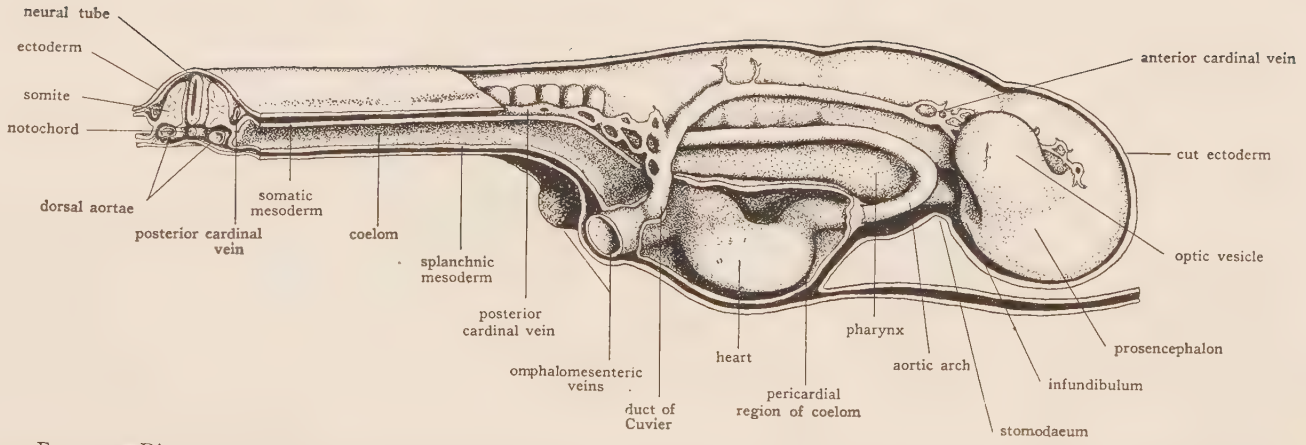


FIG. 42.—Diagrammatic lateral view of dissection of a 38-hour chick. The lateral body wall on the right side has been removed to show the internal structures. Note especially the relations of the pericardial region to that part of the cœlom which lies farther caudally, and the small anastomosing channels of the developing posterior cardinal vein from which a single main vessel is later derived.

characteristic of the adult brain are already beginning to appear. In the establishment of the five-vesicle condition of the brain, the prosencephalon is subdivided to form the telencephalon and diencephalon, the mesencephalon remains undivided, and the rhombencephalon divides to form the metencephalon and myelencephalon.

The division of the prosencephalon into telencephalon and diencephalon is not completed until a much later stage of development, but the median enlargement at this stage extending anteriad beyond the level of the optic vesicles indicates where the telencephalon will be established (Fig. 38, *D*). The optic vesicles and that part of the prosencephalon lying between them go into the diencephalon.

The mesencephalon, as stated above, undergoes no subdivision. The original mesencephalic region of the three-vesicle brain gives rise to the mesencephalon of the adult. This region of the brain does not undergo any marked differentiation until relatively late in development.

At this stage the division of the rhombencephalon is clearly marked (Fig. 38, *D* and Fig. 40). The two most anterior neuromeres of the original rhombencephalon form the metencephalon and the posterior four neuromeres are incorporated in the myelencephalon.

The Auditory Pits.—As is the case with the central nervous system, the organs of special sense arise early in development. The appearance of the optic vesicles which later become the sensory part of the eyes has already been noted. The first indication of the formation of the sensory part of the ear becomes evident at about 35 hours of incubation. At this age a pair of thickenings termed the auditory placodes arise in the superficial ectoderm of the head. They are situated on the dorso-lateral surface opposite the most posterior inter-neuromeric constriction of the myelencephalon. By 38 hours of incubation (Fig. 40) the auditory placodes have become depressed below the general level of the ectoderm and form the walls of a pair of cavities, the auditory pits. When first formed the walls of the auditory pits are directly continuous with the superficial ectoderm, and their cavities are widely open to the outside. In later stages the openings into the pits become narrowed and finally closed so that the pits become

vesicles lying between the superficial ectoderm and the myelencephalon. As yet they have no connection with the central nervous system.

The Formation of Extra-embryonic Blood Vessels.—In dealing with the circulation of the chick we must recognize at the outset two distinct circulatory arcs of which the heart is the common center. One complete circulatory arc is established entirely within the body of the embryo. A second arc is established which has a rich plexus of terminal vessels located in the extra-embryonic membranes enveloping the yolk. These are the vitelline vessels. The vitelline vessels communicate with the heart over main vessels which traverse the embryonic body. The chief distribution of the vitelline circulation is, however, extra-embryonic. Later in development there arises a third circulatory arc involving another set of extra-embryonic vessels in the allantois, but with that we have no concern until we take up later stages. Neither the intra-embryonic, nor the vitelline circulatory channels have as yet been completed but the heart and many of the main vessels have made their appearance.

The formation of extra-embryonic blood vessels is presaged by the appearance of blood islands in the vascular area of chicks toward the end of the first day of incubation (see Chapter VII). Figure 43 shows the differentiation of blood islands to form primitive blood corpuscles and blood vessels. At their first appearance the blood islands are irregular clusters of mesoderm cells lying in intimate contact with the yolk-sac entoderm (Fig. 43, *A*). When the lateral mesoderm becomes split, forming the somatic and splanchnic layers with the cœlom between, the blood islands lie in the splanchnic mesoderm adjacent to the entoderm. In embryos of 3 to 5 somites, fluid-filled spaces begin to appear in the blood islands with the result that in each blood island the peripheral cells are separated from the central ones (Fig. 43, *B*). As the fluid accumulates and the spaces expand, the peripheral cells become flattened and pushed outward, but they remain adherent to each other and completely enclose the central cells. At this stage the single layer of peripheral cells may be regarded as constituting the endothelial wall of a primitive blood channel (Fig. 43, *C*). Extension and anastomosis of neighboring blood islands which have

undergone similar differentiation results in the establishment of a network of communicating vessels. Meanwhile the cells enclosed in the primitive blood channels have become separated from each other and rounded. They soon come to contain hæmoglobin and constitute the primitive blood corpuscles.

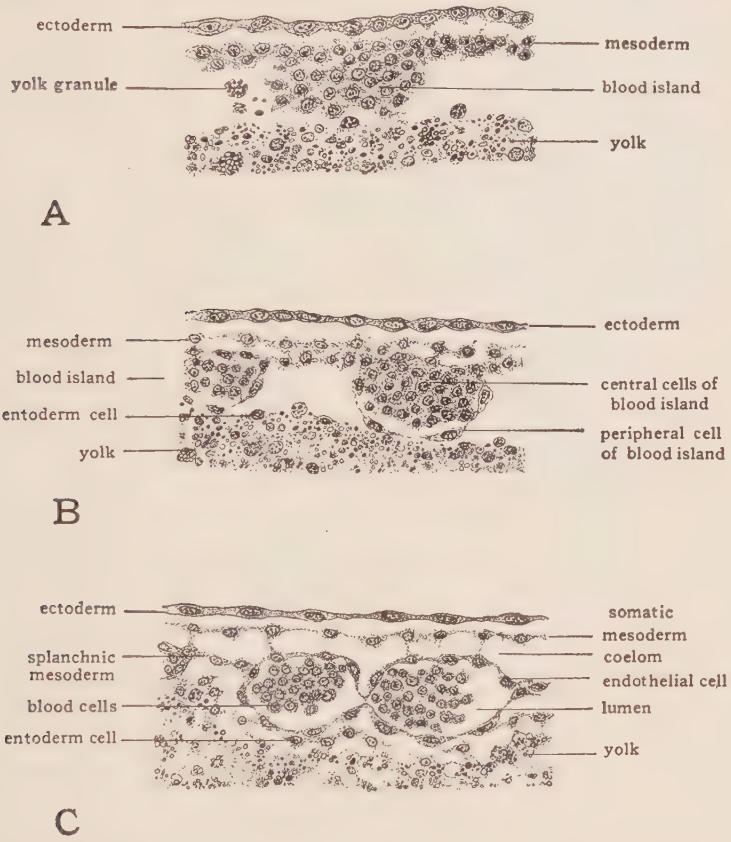


FIG. 43.—Drawings to show the cellular organization of blood islands at three stages in their differentiation. By referring to Fig. 35, *D*, the small areas here represented can be located in relation to the structure of the embryo as a whole.

A, from blastoderm of 18-hour chick; *B*, from blastoderm of 24-hour chick; *C*, from blastoderm of 33-hour chick.

The fluid accumulated in the blood islands serves as a vehicle in which the corpuscles are suspended and conveyed along the vessels.

The differentiation of the blood islands in the manner described begins first in the peripheral part of the area vasculosa and from there extends toward the body of the embryo. By

33 hours of incubation the extra-embryonic vascular plexus has extended inward and made connection with the omphalomesenteric veins which, originating within the body of the embryo have grown outward. Thus are established the afferent vitelline channels (Fig. 39).

The efferent vitelline channels have not yet appeared and there is no circulation of the blood corpuscles which are being formed in the area vasculosa. The intra-embryonic blood vessels remain empty until the extra-embryonic circuit is completed. The embryo meanwhile draws its nutrition from the yolk by direct absorption.

The Formation of the Heart.—The structural relations of the heart and the way in which it is derived from the mesoderm can be grasped only by the careful study of sections through the heart region in several stages of development (Fig. 44). The fact that the heart, itself an unpaired structure, arises from paired primordia which at first lie widely separated on either side of the mid-line, is likely to be troublesome unless its significance is understood at the outset. The paired condition of the heart at the time of its origin is due to the fact that the early embryo lies open ventrally, spread out on the yolk surface. The rudiments of all ventral structures which appear at an early age are thus at first separated, and lie on either side of the mid-line.

As the embryo develops, a series of foldings undercut it and separate it from the yolk. This folding off process at the same time establishes the ventral wall of the gut and the ventral body wall of the embryo by bringing together in the mid-line the structures formerly spread out to right and left. The primordia of the heart arise in connection with layers which are destined to form ventral parts of the embryo, but at a time when these layers are still spread out on the yolk. As the embryo is completed ventrally the paired primordia of the heart are brought together in the mid-line and become fused (Figs. 44 and 45).

The first indication of heart formation is to be seen in transverse sections passing through a 25-hour chick immediately caudal to the anterior intestinal portal. Where the splanchnopleure of either side bends toward the mid-line along the lateral margin of the intestinal portal there is a marked regional thickening in the splanchnic mesoderm of either side (Figs. 34, 44, *A*,

and 45, *A*). This pair of thickenings indicates where there has been rapid cell proliferation preliminary to the differentiation of the heart. Loosely associated cells can already be seen somewhat detached from the mesial face of the mesoderm layer. These cells soon become organized to form the endocardial primordia.

In a chick of about 26 hours, sections through a corresponding region show distinct differentiation of the endocardial and epi-myocardial primordia (Fig. 44, *B*). The endocardial primordia are a pair of delicate tubular structures, a single cell in thickness, lying between the entoderm and mesoderm. They arise from the cells seen separating from the adjacent thickened mesoderm in the 25-hour chick. As their name indicates they are destined to give rise to the endothelial lining of the heart. By far the greater part of each of the original mesodermic thickenings becomes applied to the lateral aspects of the endocardial tubes as the epi-myocardial primordium which is destined to give rise to the external coat of the heart (epicardium) and to the heavy muscular layers of the heart (myocardium).

In chicks of 27 hours the lateral margins of the anterior intestinal portal have been undergoing concrescence thus lengthening the fore-gut caudally and elongating the pericardial region. In this process the former lateral margins of the portal swing in to meet each other and fuse in the mid-line, and the endocardial tubes of the right and left side are brought toward each other beneath the newly completed floor of the fore-gut (Figs. 44, *C* and 45, *B*). In the 28-hour chick the endocardial primordia are approximated to each other (Figs. 44, *D* and 45, *C*) and by 29 hours they fuse in their mid-region to form a single tube (Figs. 44, *E* and 45, *D*).

At the same time the epi-myocardial areas of the mesoderm are brought together first ventrally (Fig. 44, *D*) and then dorsally to the endocardium (Fig. 44, *E*). Where the splanchnic mesoderm of the opposite sides of the body comes together dorsal and ventral to the heart it forms double-layered supporting membranes called respectively the dorsal mesocardium and the ventral mesocardium. The ventral mesocardium is a transitory structure, disappearing almost as soon as it is formed (Fig. 44, *E*). The dorsal mesocardium, although the greater part of it disappears in the next few hours of incubation, persists in

embryos of the stage under consideration, suspending the heart in the pericardial region of the cœlom. Conditions reached in

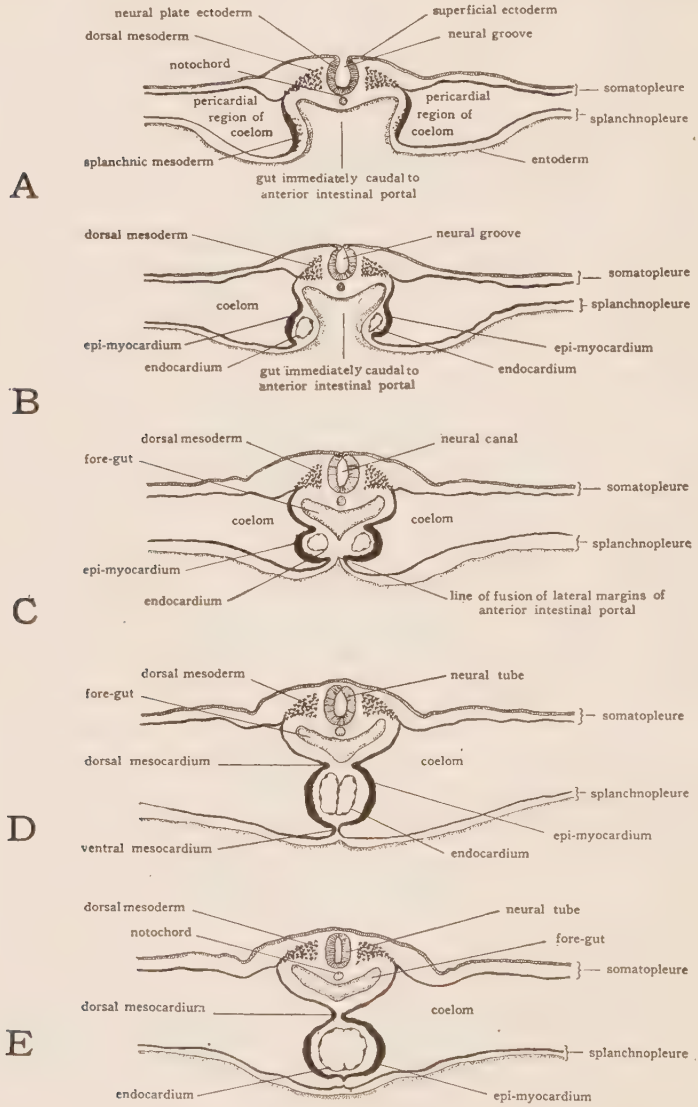


FIG. 44.—Diagrams of transverse sections through the pericardial region of chicks at various stages to show the formation of the heart. For location of the sections consult Fig. 45.

A, at 25 hours; *B*, at 26 hours; *C*, at 27 hours; *D*, at 28 hours; *E*, at 29 hours.

the heart region at 33 hours of incubation are shown in section in figure 46, *C*. The heart here is enlarged and displaced

somewhat to the right of the mid-line but its fundamental relations are otherwise the same as in a 29-hour embryo (Fig. 44, *E*).

The gross shape of the heart and its positional relations to other structures are best seen in entire embryos. The fusion

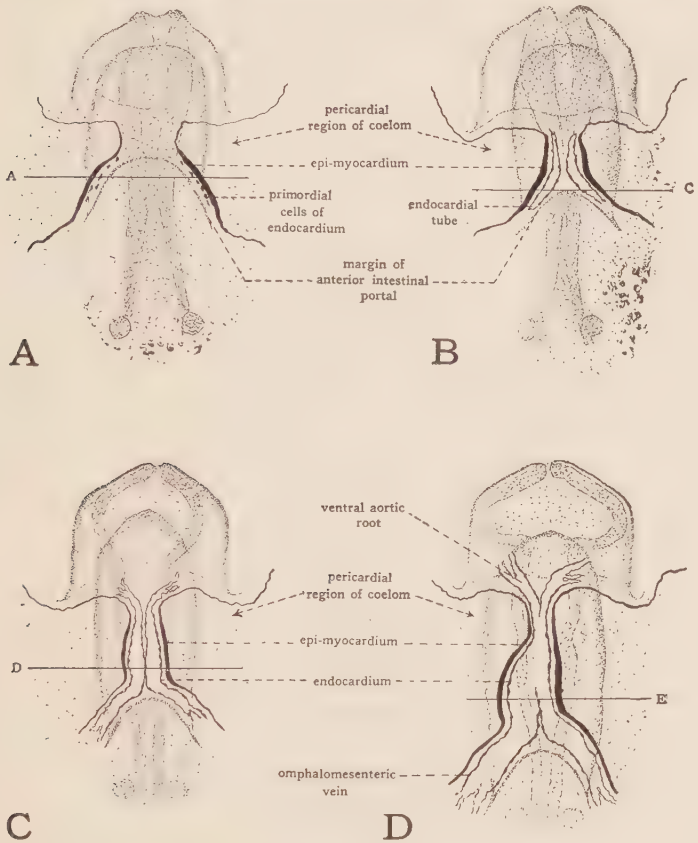


FIG. 45.—Ventral-view diagrams to show the origin and subsequent fusion of the paired primordia of the heart. The lines *A*, *C*, *D*, and *E* indicate the planes of the sections diagrammed in Fig. 44, *A*, *C*, *D*, *E*, respectively.

A, chick of 25 hours; *B*, chick of 27 hours; *C*, chick of 28 hours; *D*, chick of 29 hours.

of the paired cardiac primordia establishes the heart as a nearly straight tubular structure. It lies at the level of the rhombencephalon in the mid-line, ventral to the fore-gut (Fig. 37). By 33 hours of incubation the mid-region of the heart is considerably dilated and bent to the right (Fig. 39). At 38 hours the heart is bent so far to the right that it extends

beyond the lateral body margin of the embryo (Fig. 40). This bending process is correlated with the rupture of the dorsal mesocardium at the mid-region of the heart. The breaking through of the dorsal and ventral mesocardia is of interest aside from the fact that it leaves the heart free to undergo changes in shape. It makes the right and left cœlomic chambers confluent, the pericardial region thus being the first part of the cœlom to acquire the unpaired condition characteristic of the adult.

Although there are as yet no sharply bounded subdivisions of the heart, it is convenient to distinguish three regions which later become clearly marked off from each other (Fig. 41). The most caudal part of the heart where the omphalomesenteric veins join is the sino-atrial region; the part of the heart which is dilated and bent to the right will become the ventricular region; and the region where the ventricle swings into the midline and becomes narrowed is known as the bulbo-conus arteriosus. Approximately at the stage of development indicated in figure 41 irregular twitchings occur in the heart walls, but regular pulsations are not established until about the 40th to the 44th hour of incubation.

The Formation of the Intra-embryonic Blood Vessels.—Concurrently with the establishment of the heart, blood vessels have arisen within the body of the embryo. Concerning the exact nature of the process of blood vessel formation there has been much disagreement. The weight of evidence now indicates that the early vessels are formed from mesodermal cells which lie in the path of their development. They grow by organization of cells in situ as a drain might be built from bricks already deposited along its projected course. In later stages it seems probable that vessels extend by the formation of bud-like outgrowths from their walls, as well as by organization of cells in their path of development. When first formed, the blood vessel walls are but a single cell in thickness. There is no structural differentiation between arteries and veins until a considerably later period. Recognition of the vessels depends wholly, therefore, on determining their course and relationships.

The large vessels connecting with the heart are the first of the intra-embryonic channels established. From the bulbo-

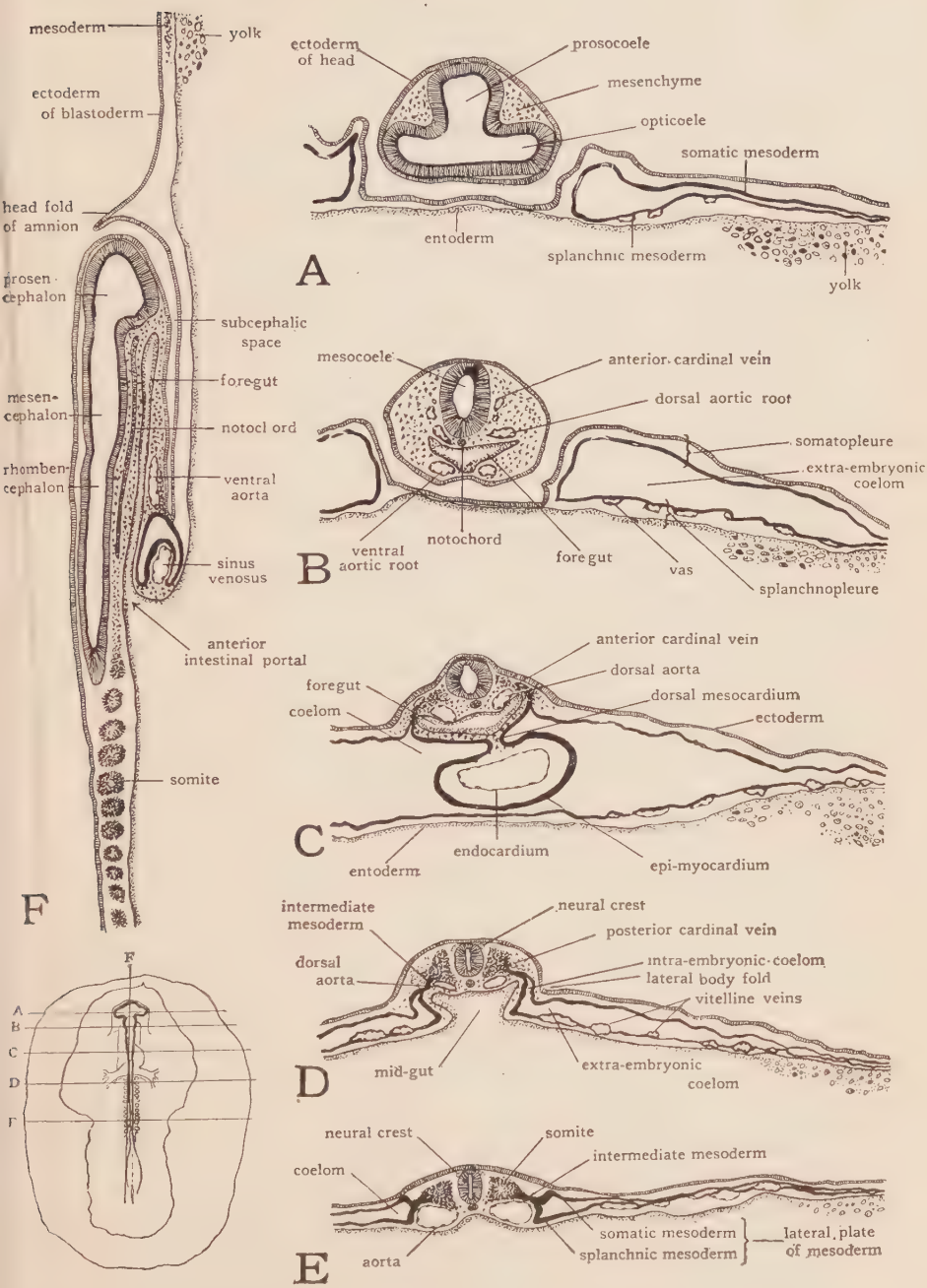


FIG. 46.—Diagrams of sections of 33-hour chick. The location of each section is indicated on a small outline sketch of the entire embryo.

conus arteriosus the paired ventral aortic roots extend cephalad, ventral to the fore-gut (Fig. 41). At the cephalic end of the fore-gut the ventral aortic roots turning dorsad curve around it, and then extend caudad, dorsal to the gut, as the paired dorsal aortæ (Figs. 41, 42 and Fig. 46, *B*). Few conspicuous branches arise from the aortæ at this stage but as development progresses branches extend to the various parts of the embryo and the aortæ become the main efferent conducting vessels of the embryonic circulation. Both the ventral aortic roots and the omphalomesenteric veins are direct continuations of the paired endocardial primordia of the heart. The epi-myocardial coat is formed about the original endothelial tubes only where they are fused in the region destined to become the heart. The development of the heart at this stage is an epitome of its phylogenetic origin. The local investment of the endocardial tubes by the epi-myocardium, as seen in the formation of the chick heart, is a recapitulation of the evolutionary origin of the heart by the local addition of a heavy muscular coat about the walls of a blood vessel.

During early embryonic life the cardinal veins are the main afferent vessels of the intra-embryonic circulation. The main cardinal trunks are paired vessels symmetrically placed on either side of the mid-line. There are two pairs, the anterior cardinals which return the blood to the heart from the cephalic region of the embryo, and the posterior cardinals which return the blood from the caudal region. The anterior and posterior cardinal veins of the same side of the body become confluent dorsal to the level of the heart. The vessels formed by the junction of the anterior and posterior cardinals are the ducts of Cuvier or common cardinal veins. The right and left ducts of Cuvier turn ventrad, one on either side of the fore-gut, and enter the sinus-venosus along with the right and left omphalomesenteric veins, respectively (Fig. 42).

In chicks of 33 hours the anterior cardinal veins can usually be made out in sections (Fig. 46, *B*, *C*). By 38 hours the anterior cardinals and the ducts of Cuvier are readily recognized The posterior cardinals appear somewhat later than the anterior cardinals but are ordinarily discernible in the region of the duct of Cuvier by 33 to 35 hours and well established by 38 hours. For the sake of simplicity and clearness the cardinal

veins have been represented in figure 42 larger and more regularly formed than they are in actual specimens. Like all the other blood vessels of the embryo they arise as irregular anastomosing endothelial tubes, only gradually taking on the regularity of shape characteristic of fully formed vessels.

CHAPTER X

THE CHANGES BETWEEN FORTY AND FIFTY HOURS OF INCUBATION

FLEXION AND TORSION; THE COMPLETION OF THE VITELLINE CIRCULATORY CHANNELS; THE BEGINNING OF THE CIRCULATION OF BLOOD.

Flexion and Torsion.—Until 36 or 37 hours of incubation the longitudinal axis of the chick is straight except for slight fortuitous variations. Beginning at about 38 hours, processes are initiated which eventually change the entire configuration of the embryo and its positional relations to the yolk. These processes involve positional changes of two distinct types, flexion and torsion. As applied to an embryo, flexion means the bending of the body about a transverse axis, as one might bend the head forward at the neck, or the trunk forward at the hips. Torsion means the twisting of the body, as one might turn the head and shoulders in looking backwards without changing the position of the feet.

In chick embryos the first flexion of the originally straight body-axis takes place in the head region. Because of its location it is known as the cranial flexure. The axis of bending in the development of the cranial flexure is a transverse axis passing through the mid-brain at the level of the anterior end of the notochord. The direction of the flexion is such that the fore-brain becomes bent ventrally toward the yolk. The process is carried out as if the brain were being bent about the anterior end of the notochord. Until the cranial flexure is well established it is inconspicuous in dorsal views of whole-mounts but even in its initial stages it appears plainly in lateral views (Fig. 42).

To appreciate the correlation between the processes of flexion and torsion it is only necessary to bear in mind the relation of a chick of this age to the yolk. As long as the chick lies with its ventral surface closely applied to the yolk, the yolk consti-

tutes a bar to flexion. Before extensive flexion can be carried out the chick must twist around on its side, *i.e.*, undergo torsion, as a man lying face down turns on his side in order to flex his body.

Torsion begins in the cephalic region of the embryo and progresses caudad. The first indications of torsion appear almost as soon as the cranial flexure begins and the two processes then progress synchronously. In the chick, torsion is normally car-

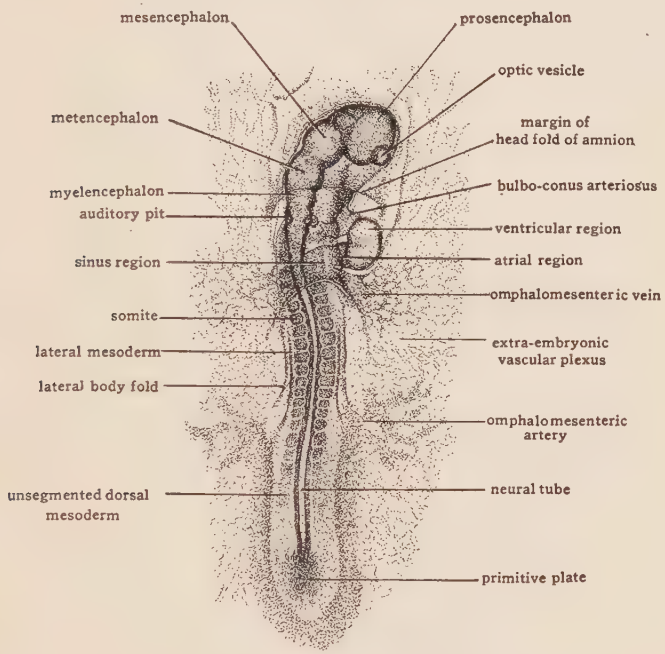


FIG. 47.—Dorsal view (× 14) of entire chick embryo having 19 pairs of somites (about 43 hours incubation). Due to torsion the cephalic region appears in dextro-dorsal view.

ried out toward a definite side. The cephalic region of the embryo is twisted in such a manner that the left side comes to lie next to the yolk and the right side away from the yolk. The progress of torsion caudad is gradual and the posterior part of the embryo remains prone on the yolk for a considerable time after torsion has been completed in the head region. Figure 40 shows the head of an embryo of about 38 hours in which the cranial flexure and torsion are just becoming evident. In chicks of about 43 hours (Fig. 47) the further progress of both flexion and torsion is well marked.

The processes of flexion and torsion thus initiated continue until the original orientation of the chick on the yolk is completely changed. As the body of the embryo becomes turned on its side the yolk no longer impedes the progress of flexion. Following the accomplishment of torsion in the cephalic region, the cranial flexure becomes rapidly greater until the head is practically doubled on itself (Fig. 55). As development proceeds, torsion progresses caudad involving more and more of the body of the embryo. Finally the entire embryo comes to lie with its left side on the yolk. Concomitantly with the progress of torsion, flexion also appears farther caudally, affecting in turn the cervical dorsal, caudal regions. The series of flexions which accompany torsion bend the head and tail of the embryo ventrally so that its spinal axis becomes C-shaped (Fig. 64). The flexions which bend the embryo on itself so the head and tail lie close together are characteristic not of the chick alone, but of the embryos of reptiles, birds, and mammals generally. Flexion would seem to be correlated with the spatial limitations of the egg or the uterine cavity within which these embryos undergo their development. Certainly there is no appreciable flexion in embryos of fishes and amphibia which develop free in water with no imposed limitations of space. The torsion which in the chick accompanies flexion, is characteristic only of embryos developing on the surface of a very large yolk which would act as an impediment to flexion unless the embryo had first turned on its side.

The Completion of the Vitelline Circulatory Channels.—In chicks of 33 to 36 hours the omphalomesenteric veins have been established as postero-lateral extensions of the same endocardial tubes which are involved in the formation of the heart. As the omphalomesenteric veins are extending laterad, the vessels developing in the vitelline plexus are extending and converging toward the embryo. Eventually the vitelline vessels attain communication with the heart by becoming confluent with the omphalomesenteric veins. This establishes the afferent[1] channels of the vitelline circulation.

[1] In dealing with vascular channels the term afferent means that the blood is flowing toward the heart, and efferent means that the blood is flowing away from the heart. Thus when we speak of the afferent channels of the vitelline circulation the dominant idea in our mind is not the vitelline plexus as a local center of vascular activity, but the heart as the common pumping center for the entire

The vessels destined to carry blood from the heart to the vitelline plexus develop in embryos of about 40 hours (Fig. 47). Like the afferent vitelline channels, the efferent channels have

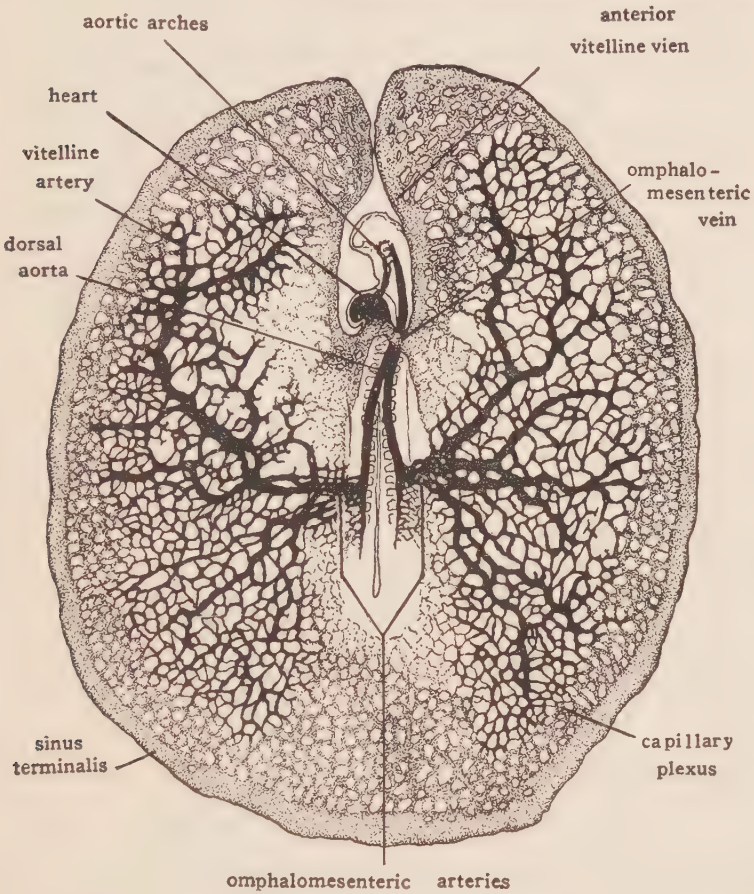


FIG. 48.—The vitelline circulation in a chick of about 44 hours incubation. Diagrammatic ventral view based on Popoff's figures of injected embryos. The arteries are shown in solid black and the veins are stippled. Note the rich plexus of small freely anastomosing vessels in the splanchnopleure of the yolk-sac.

a dual origin. The proximal portions of the efferent channels arise within the embryo as branches of the dorsal aortæ, and

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circulatory mechanism. The statement that the omphalomesenteric vein *is the main afferent vessel of the vitelline circulation* can be paraphrased:—the omphalomesenteric vein *is the main channel through which the blood, having passed through the small peripherally located vessels of the vitelline plexus, is carried back to the heart.*

extend peripherally. The distal portions of the channels arise in the extra-embryonic vascular area and extend toward the embryo. The efferent vitelline circulation cannot begin until these two sets of channels become confluent. At first their connection with each other is by way of a network of small channels rather than by large vessels. These preliminary channels are formed as exceedingly small, freely anastomosing vessels extending from the aorta to communicate laterally with the extra-embryonic plexus. Later some of these channels become confluent, others disappear, and gradually definite main vessels, the omphalomesenteric arteries, are established. For some time after their formation, the omphalomesenteric arteries are likely to retain traces of their origin from a plexus of small channels and arise from the aorta by several roots (Fig. 56).

The Beginning of the Circulation of Blood.—At about 44 hours of incubation, coincidentally with the completion of the vitelline vessels, the heart begins regular contraction, and the blood which has been formed in the extra-embryonic vascular area is for the first time pumped through the vessels of the embryo. In tracing the course of either the embryonic or the vitelline circulation the heart is the logical starting point. From the heart the blood of the extra-embryonic vitelline circulation passes through the ventral aortæ, thence through the aortic arches and along the dorsal aortæ, and out through the omphalomesenteric arteries to the plexus of vessels on the yolk (Fig. 48).

In the small vessels which ramify in the membranes enveloping the yolk the blood absorbs food material. In young embryos, before the allantoic circulation has appeared, the vitelline circulation is involved also in the oxygenation of the blood. The great surface exposure presented by the multitude of small vessels on the yolk makes it possible for the blood to take up oxygen which penetrates the porous shell and the albumen.

After acquiring food material and oxygen the blood is collected by the sinus terminalis and the vitelline veins. The vitelline veins converge toward the embryo from all parts of the vascular area and empty into the omphalomesenteric veins which return the blood to the heart (Figs. 48 and 77).

The blood of the intra-embryonic circulation, leaving the heart enters the ventral aortæ, thence passes by way of the aortic arches into the dorsal aortæ, and is distributed through branches from the dorsal aortæ to the body of the embryo. It is returned from the cephalic part of the body by the anterior cardinals, and from the caudal part of the body by the posterior cardinals. The anterior and posterior cardinals discharge together through the ducts of Cuvier into the sinus region of the heart (Fig. 42).

In the heart, the blood of the extra-embryonic circulation and of the intra-embryonic circulation is mixed. The mixed blood in the heart is not as rich in oxygen and food material as that which comes to the heart from the vitelline circulation nor as low in food and oxygen content as that returned to the heart from the intra-embryonic circulation where these materials are drawn upon by the growing tissues of the embryo. Nevertheless it carries a sufficient proportion of food and oxygen so that as it is distributed to the body of the embryo it serves to supply the growing tissues.

CHAPTER XI

EXTRA-EMBRYONIC MEMBRANES

THE FOLDING OFF OF THE BODY OF THE EMBRYO; THE ESTABLISHMENT OF THE YOLK-SAC AND THE DELIMITATION OF THE EMBRYONIC GUT; THE AMNION AND THE SEROSA; THE ALLANTOIS.

The Folding off of the Body of the Embryo.—In bird embryos the somatopleure and splanchnopleure extend over the yolk peripherally, beyond the region where the body of the embryo is being formed. Distal to the body of the embryo the layers are termed extra-embryonic. At first the body of the chick has no definite boundaries and consequently embryonic and extra-embryonic layers are directly continuous without there being any definite boundary at which we may say one ends and the other begins. As the body of the embryo takes form, a series of folds develop about it, undercut it, and finally nearly separate it from the yolk. The folds which thus definitely establish the boundaries between intra-embryonic and extra-embryonic regions are known as the limiting body folds or simply the body folds.

The first of the body folds to appear is the fold which marks the boundary of the head. By the end of the first day of incubation the head has grown anteriorly and the fold originally bounding it appears to have undercut and separated it anteriorly from the blastoderm (Figs. 2 and 32). The cephalic limiting fold at this stage is crescentic, concave caudally. As this fold continues to progress caudad, its posterior extremities become continuous with folds which develop along either side of the embryo. Because of the fact that these folds bound the body of the embryo laterally, they are known as the lateral body folds (lateral limiting sulci). The lateral body folds, at first shallow (Fig. 46, *D*) become deeper, undercutting the body of the embryo from either side and further separating it from the yolk (Fig. 57, *E* and Fig. 49).

During the third day a fold appears bounding the posterior region of the embryo (Fig. 50, *C*). This caudal fold undercuts the tail of the embryo forming a sub-caudal pocket just as the sub-cephalic fold undercuts the head. The combined effect of the development of the sub-cephalic, lateral body, and the sub-caudal folds is to constrict off the embryo more and more from the yolk (Figs. 49 and 52). These folds which establish the contour of the embryo indicate at the same time the boundary between the tissues which are built into the body of the embryo, and the so-called extra-embryonic tissues which serve temporary purposes during development but are not incorporated in the structure of the adult body.

The Establishment of the Yolk-sac and the Delimitation of the Embryonic Gut.—The extra-embryonic membranes of the chick are four in number, the yolk-sac, the amnion, the serosa, and the allantois. The yolk-sac is the first of these to make its appearance. The splanchnopleure of the chick instead of forming a closed gut, as happens in forms with little yolk, grows over the yolk surface. The primitive gut has a cellular wall dorsally only, while the yolk acts as a temporary floor (Fig. 50, *A*). The extra-embryonic extension of the splanchnopleure eventually forms a sac-like investment for the yolk, (Figs. 49 and 52).

Concomitantly with the spreading of the extra-embryonic splanchnopleure about the yolk, the intra-embryonic splanchnopleure is undergoing a series of changes which result in the establishment of a completely walled gut in the body of the embryo. The interrelations of the various steps in the formation of the gut and of the yolk-sac make it necessary to repeat some points and anticipate other points concerning the formation of the gut, in order that their relation to yolk-sac formation may not be overlooked.

It will be recalled that the first part of the primitive gut to acquire a cellular floor is its cephalic region. The same folding process by which the head is separated from the blastoderm involves the entoderm of the gut. The part of the primitive gut which acquires a floor as the sub-cephalic fold progresses caudad is termed the fore-gut (Fig. 50, *B*). During the third day of incubation the caudal fold undercuts the posterior end of the embryo. The splanchnopleure of the gut is involved

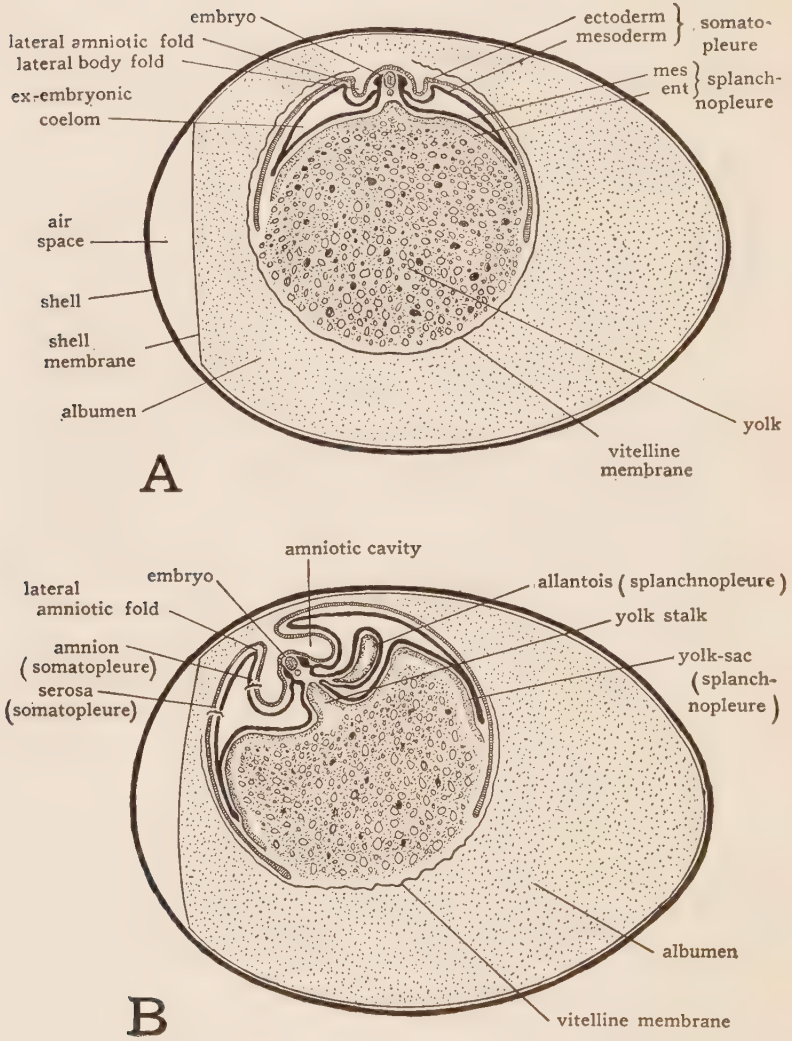

embryo
lateral amniotic fold
lateral body fold
ex-embryonic coelom
air space
shell
shell membrane
albumen
ectoderm
mesoderm
somato-pleure
mes
ent
splanch-nopleure
yolk
vitelline membrane
A
amniotic cavity
embryo
lateral amniotic fold
amnion (somatopleure)
serosa (somatopleure)
allantois (splanchnopleure)
yolk stalk
yolk-sac (splanch-nopleure)
albumen
vitelline membrane
B

FIG. 49.

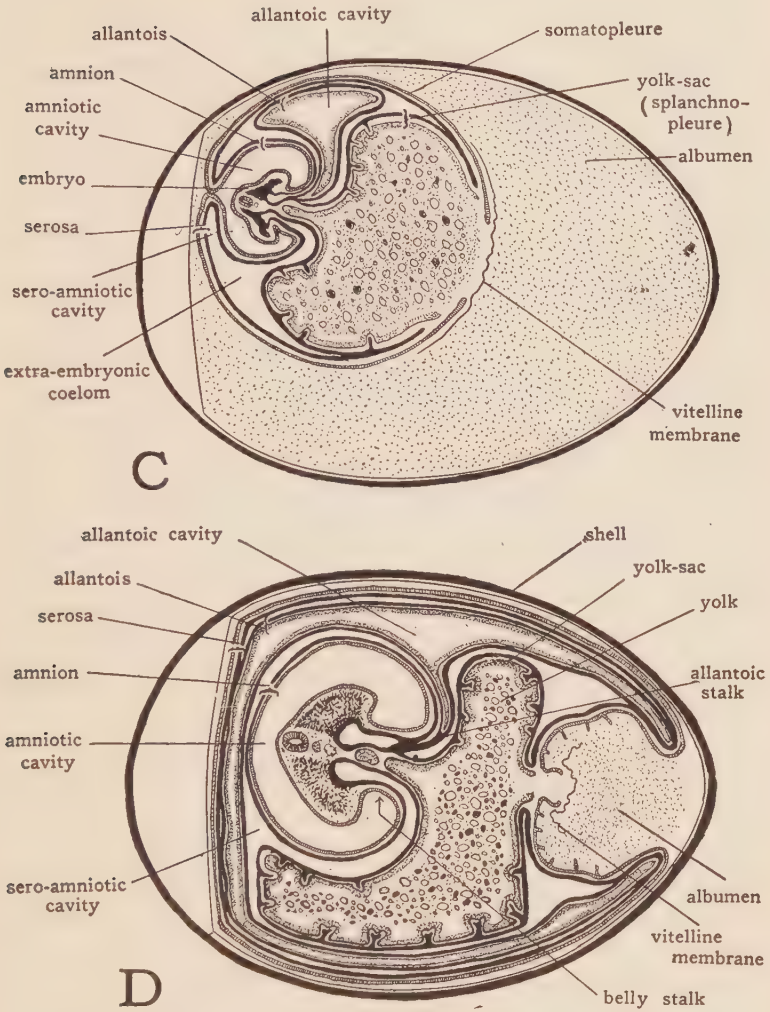


FIG. 49.—Schematic diagrams to show the extra-embryonic membranes of the chick. (*After Duval.*) The diagrams represent longitudinal sections through the entire egg. The body of the embryo, being oriented approximately at right angles to the long-axis of the egg, is cut transversely.

A, embryo of about two days incubation; *B*, embryo of about three days incubation; *C*, embryo of about five days incubation; *D*, embryo of about fourteen days incubation.

in the progress of the sub-caudal fold so that a hind-gut is established in a manner analogous to the formation of the fore-gut (Fig. 50, *C*). The part of the gut which still remains open to the yolk is known as the mid-gut. As the embryo is constricted off from the yolk by the progress of the sub-cephalic and sub-caudal folds, the fore-gut and hind-gut are increased in extent at the expense of the mid-gut. The mid-gut is finally diminished until it opens ventrally by a small aperture which flares out, like an inverted funnel, into the yolk-sac (Fig. 50, *D*). This opening is the yolk-duct and its wall constitutes the yolk-stalk.

The walls of the yolk-sac are still continuous with the walls of the gut along the constricted yolk-stalk thus formed, but the boundary between the intra-embryonic splanchnopleure of the gut and the extra-embryonic splanchnopleure of the yolk-sac can now be established definitely at the yolk-stalk.

As the neck of the yolk-sac is constricted the omphalomesenteric arteries and omphalomesenteric veins, caught in the same series of foldings, are brought together and traverse the yolk-stalk side by side. The vascular network in the splanchnopleure of the yolk-sac, which in young chicks was seen spreading over the yolk, eventually nearly encompasses it. The embryo's store of food material thus comes to be suspended from the gut of the mid-body region in a sac provided with a circulatory arc of its own, the vitelline arc. Apparently no yolk passes directly through the yolk-duct into the intestine. Absorption of the yolk is effected by the epithelium of the yolk-sac and the food material is transferred to the embryo by the vitelline circulation. In older embryos (Fig. 49, *C* and *D*) the epithelium of the yolk-sac undergoes a series of foldings which greatly increase its surface area and thereby the amount of absorption it can accomplish.

During development the albumen loses water, becomes more viscid, and rapidly decreases in bulk. The growth of the allantois, an extra-embryonic structure which we have yet to consider, forces the albumen toward the distal end of the yolk-sac (Fig. 49, *D*). The manner in which the albumen is encompassed between the yolk-sac and folds of the allantois and serosa belong to later stages of development than those with which we are concerned. Suffice it to say that the albumen,

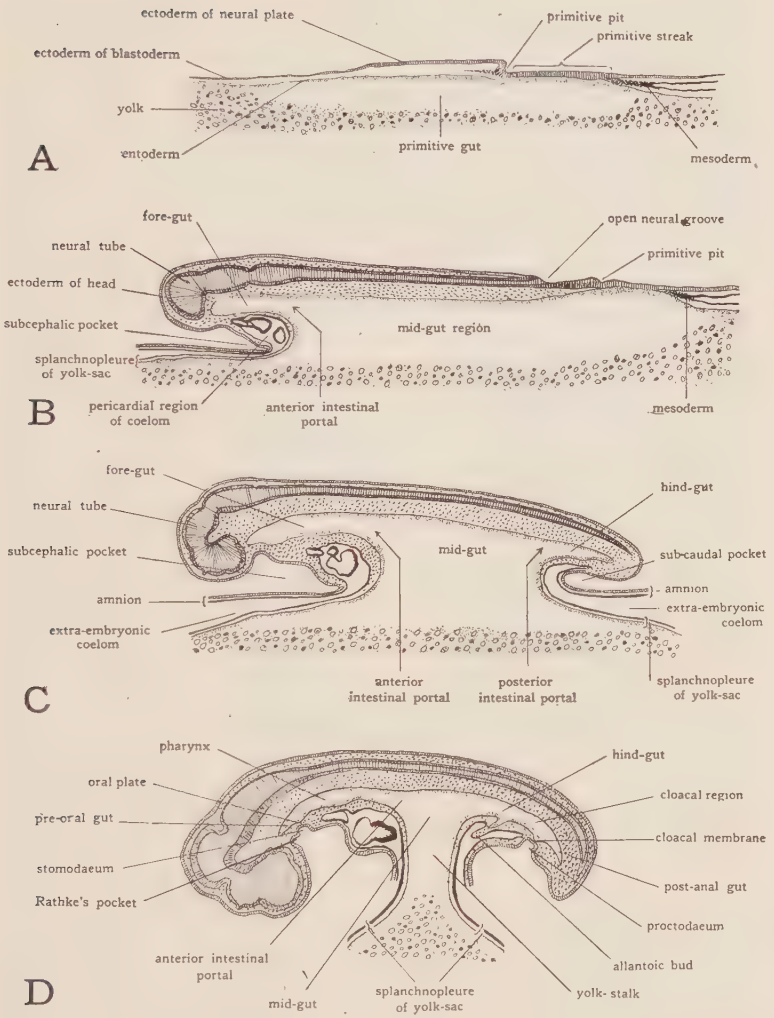


FIG. 50.—Schematic longitudinal-section diagrams of the chick showing four stages in the formation of the gut tract. The embryos are represented as unaffected by torsion.

A, chick toward the end of the first day of incubation; no regional differentiation of primitive gut is as yet apparent. *B*, toward the end of the second day; fore-gut established. *C*, chick of about three days; fore-gut, mid-gut and hind-gut established. *D*, chick of about four days; fore-gut and hind-gut increased in length at expense of mid-gut; yolk-stalk formed.

FIG. 51.—Unstained chick of about 40 hours incubation photographed by reflected light to show the cephalic fold of the amnion enveloping the head of the embryo.

like the yolk, is surrounded by extra-embryonic membranes by which it is absorbed and transferred by way of the extra-embryonic circulation to the embryo.

Toward the end of the period of incubation, usually on the 19th day, the remains of the yolk-sac are enclosed within the body walls of the embryo. After its inclusion in the embryo, both the wall and the remaining contents of the yolk-sac rapidly disappear, their absorption being practically completed in the first six days after hatching.

The Amnion and the Serosa.—The amnion and the serosa are so closely associated in their origin that they must be considered together. Both are derived from the extra-embryonic somatopleure. The amnion encloses the embryo as a saccular investment and the cavity thus formed between the amnion and the embryo becomes filled with a watery fluid. Suspended in this amniotic fluid, the embryo is free to change its shape and position, and external pressure upon it is equalized. Muscle fibres develop in the amnion, which by their contraction gently agitate the amniotic fluid. The movement thus imparted to the embryo apparently aids in keeping it free and preventing adhesions and resultant malformations.

The first indication of amnion formation appears in chicks of about 30 hours incubation. The head of the embryo sinks into the yolk somewhat, and at the same time the extra-embryonic somatopleure anterior to the head is thrown into a fold, the head fold of the amnion (Fig. 52, *A*). In dorsal aspect the margin of this fold is crescentic in shape with its concavity directed toward the head of the embryo. The head fold of the amnion must not be confused with the sub-cephalic fold which arises earlier in development and undercuts the head.

As the embryo increases in length its head grows anteriorly into the amniotic fold. Growth in the somatopleure itself tends to extend the amniotic fold caudad over the head of the embryo (Fig. 52, *B*). By continuation of these two growth processes the head soon comes to lie in a double walled pocket of extra-embryonic somatopleure which covers the head like a cap (Fig. 51). The free edge of the amniotic pocket retains its original crescentic shape as, in its progress caudad, it covers more and more of the embryo (Figs. 47 and 55).

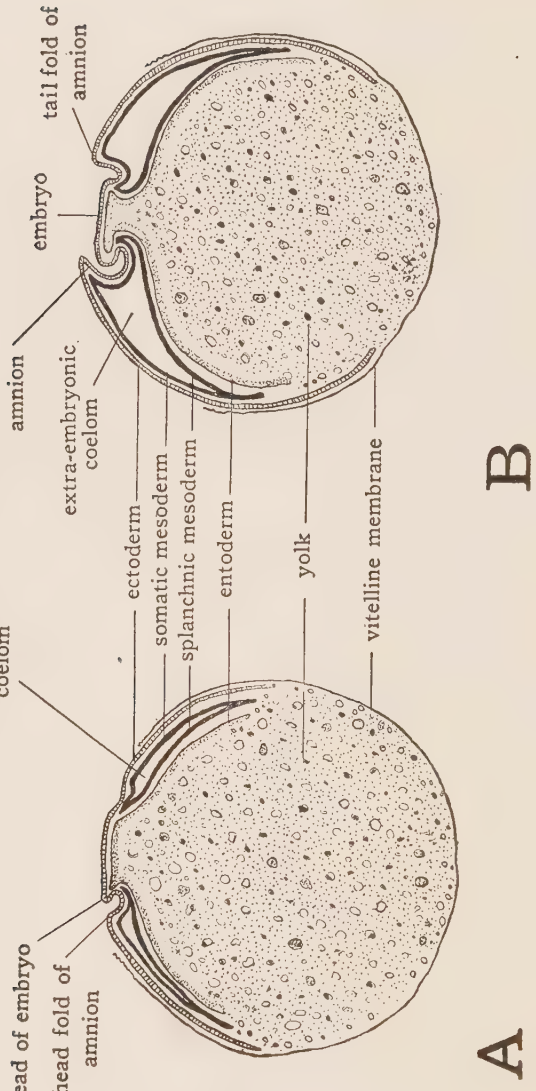

head of embryo
head fold of amnion
extra-embryonic coelom
ectoderm
somatic mesoderm
splanchnic mesoderm
entoderm
yolk
vitelline membrane
head fold of amnion
extra-embryonic coelom
embryo
tail fold of amnion
A
B

FIG 52.

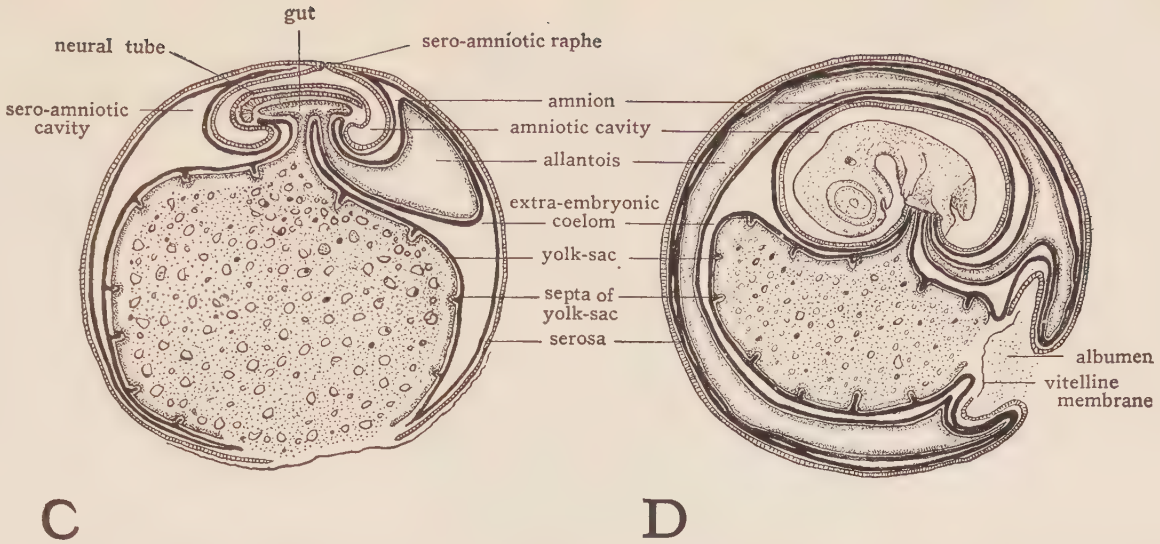


FIG. 52.—Schematic diagrams to show the extra-embryonic membranes of the chick. (*D, after Lillie.*) The embryo is cut longitudinally. The albumen, shell membranes and shell are not shown; for their relations see Fig. 49.

A, embryo early in second day of incubation; *B*, embryo early in third day of incubation; *C*, embryo of five days; *D*, embryo of nine days.

The caudally-directed limbs of the head fold of the amnion are continued posteriorly along either side of the embryo as the lateral amniotic folds. The lateral folds of the amnion grow dorso-mesiad, eventually meeting in the mid-line dorsal to the embryo (Fig. 49, *A-C*).

During the third day, the tail-fold of the amnion develops about the caudal region of the embryo. Its manner of development is similar to that of the head fold of the amnion but its direction of growth is reversed, its concavity being directed anteriorly and its progression being cephalad (Fig. 52, *B*, *C*).

Continued growth of the head, lateral, and tail folds of the amnion results in their meeting above the embryo. At the point where the folds meet, they become fused in a scar-like thickening termed the amniotic raphe (sero-amniotic raphe). (Fig. 52, *C*.) The way in which the somatopleure has been folded about the embryo leaves the amniotic cavity completely lined by ectoderm which is continuous with the superficial ectoderm of the embryo at the region where the yolk-stalk enters the body (Fig. 49, *D*).

All the amniotic folds involve doubling the somatopleure on itself. Only the inner layer of the somatopleuric fold is involved in the formation of the amniotic cavity. The outer layer of somatopleure becomes the serosa (Fig. 49, *B*). The cavity between serosa and amnion (sero-amniotic cavity) is part of the extra-embryonic cœlom. The continuity of the extra-embryonic cœlom with the intra-embryonic cœlom is most apparent in early stages (Fig. 49, *A* and *B*). They remain, however, in open communication in the yolk-stalk region until relatively late in development.

The rapid peripheral growth of the somatopleure carries the serosa about the yolk-sac, which it eventually envelops. The albumen-sac also is surrounded by folds of serosa, and the allantois, after its establishment, develops within the serosa, between it and the amnion. Thus the serosa eventually encompasses the embryo itself and all the other extra-embryonic membranes. The relationships of the serosa and allantois and the functional significance of the serosa will be taken up after the allantois has been considered.

The Allantois.—The allantois differs from the amnion and serosa in that it arises primarily within the body of the embryo. Its proximal portion is intra-embryonic throughout development. Its distal portion, however, is carried outside the confines of the intra-embryonic cœlom and becomes associated with the other extra-embryonic membranes. Like the other extra-embryonic membranes the distal portion of the allantois

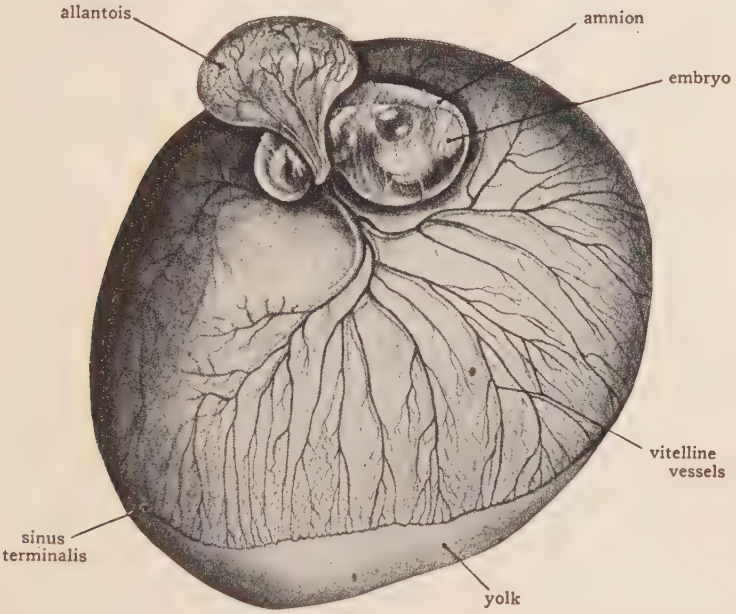


FIG. 53.—Chick of about 5½ days incubation taken out of the shell with the yolk intact. (*Modified from Kerr.*) The albumen and the serosa have been removed to expose the embryo lying within the amnion, and the allantois has been displaced upward in order to show the relations of the allantoic stalk. Compare this figure with figure 49, *C* which shows schematically the relations of the membranes in a section through an embryo of similar age.

functions only during the incubation period and is not incorporated into the structure of the adult body.

The allantois first appears late in the third day of incubation. It arises as a diverticulum from the ventral wall of the hind-gut and its walls are, therefore, splanchnopleure. Its relationships to structures within the embryo will be better understood when chicks of three and four days incubation have been studied, but its general location can be appreciated from the schematic diagrams of figures 52 and 54.

During the fourth day of development the allantois pushes out of the body of the embryo into the extra-embryonic cœlom. Its proximal portion lies parallel to the yolk-stalk and just

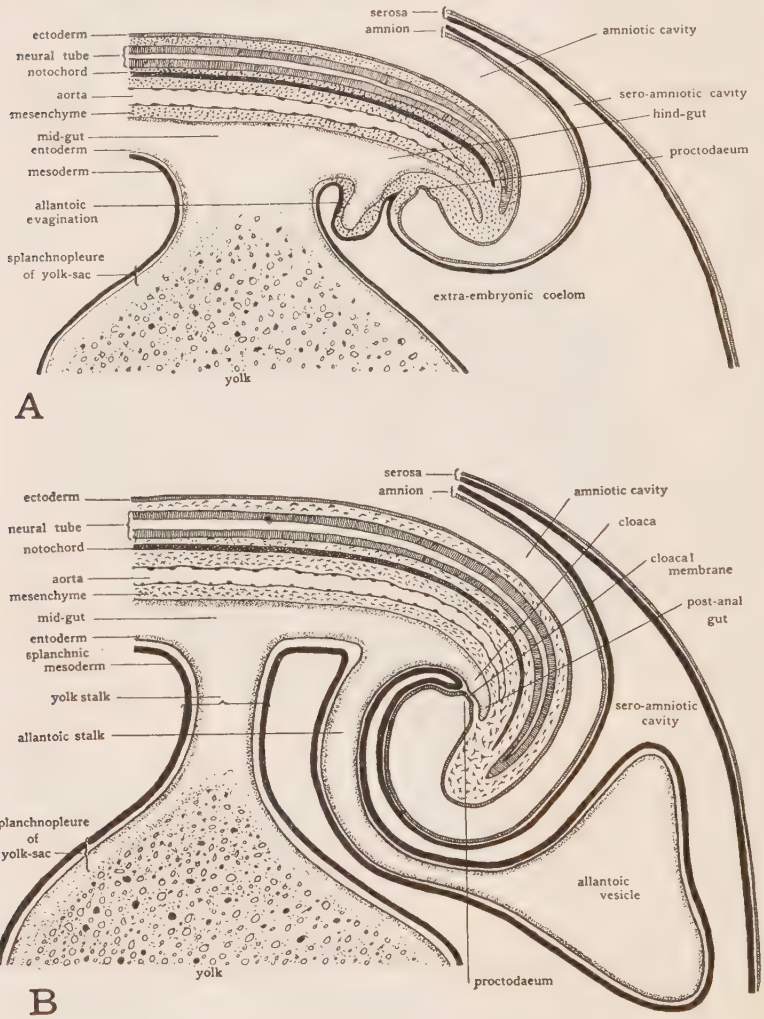


FIG. 54.—Schematic longitudinal-section diagrams of the caudal half of the embryo to show the formation of the allantois.

A, chick of about three days incubation; *B*, chick of about four days incubation.

caudal to it. When the distal portion of the allantois has grown clear of the embryo it becomes enlarged (Fig. 52, *C*). Its narrow proximal portion is known as the allantoic stalk,

the enlarged distal portion as the allantoic vesicle. Fluid accumulating in the allantois distends it so the appearance of its terminal portion in entire embryos is somewhat balloon-like (Fig. 53).

The allantoic vesicle enlarges very rapidly from the fourth to the tenth day of incubation. Extending into the sero-amniotic cavity it becomes flattened and finally encompasses the embryo and the yolk-sac (Fig. 49, *C*, *D*). In this process the mesodermic layer of the allantois becomes fused with the adjacent mesodermic layer of the serosa. There is thus formed a double layer of mesoderm, the serosal component of which is somatic mesoderm and the allantoic component of which is splanchnic mesoderm. In this double layer of mesoderm an extremely rich vascular network develops which is connected with the embryonic circulation by the allantoic arteries and veins. It is through this circulation that the allantois carries on its primary function of oxygenating the blood of the embryo and relieving it of carbon dioxide. This is made possible by the position occupied by the allantois, close beneath the porous shell (Fig. 49). In addition to its primary respiratory function the allantois serves as a reservoir for the secretions coming from the developing excretory organs and also takes part in the absorption of the albumen.

The fusion of the allantoic mesoderm and blood vessels with the serosa is of particular interest because of its homology with the establishment of the chorion in the higher mammals.[1] The chorion of mammalian embryos arises by the fusion of allantoic vessels and mesoderm with the inner wall of the serosa, and constitutes the embryos' organ of attachment to the uterine wall. In mammalian embryos the allantoic, or

[1] The serosa of the chick is occasionally called chorion. The term chorion, however, is more frequently applied to the composite layer formed by the fusion of the allantois and the serosa. The latter is a much more proper usage, since these two conjoined layers, rather than the serosa alone, correspond with the chorion of mammals. The homologies are somewhat masked by running together of the early steps in the process, and by reduction in size of the allantoic cavity, yet the mammalian chorion can be analyzed into an outer portion homologous with the serosa of the chick, and an inner portion (intimately fused to the outer from their first appearance), which represents allantoic vessels and mesoderm.

In some books the term outer or false amnion will be found used to designate the structure called in this book serosa. The term false amnion is not, however, in general use in this country.

umbilical circulation as it is usually called in mammals, serves more than a respiratory function. In the absence of any appreciable amount of yolk, the mammalian embryo derives its nutrition, through the allantoic circulation, from the uterine blood of the mother. Thus the mammalian allantoic circulation carries out the functions which in the chick are divided between the vitelline and the allantoic circulations.

CHAPTER XII

THE STRUCTURE OF CHICKS FROM FIFTY TO FIFTY-FIVE HOURS OF INCUBATION

I. External Features.
II. The Nervous System.
Growth of the telencephalic region; the epiphysis; the infundibulum and Rathke's pocket; the optic vesicles; the lens; the posterior part of the brain and the cord region of the neural tube; the neural crest.
III. The Digestive Tract.
The fore-gut; the stomodæum; the pre-oral gut; the mid-gut; the hind-gut.
IV. The Visceral Clefts and Visceral Arches.
V. The Circulatory System.
The heart; the aortic arches; the fusion of the dorsal aortæ; the cardinal and omphalomesenteric vessels.
VI. The Differentiation of the Somites.
VII. The Urinary System.

I. External Features

In chicks which have been incubated from 50 to 55 hours (Fig. 55) the entire head region has been freed from the yolk by the progress caudad of the sub-cephalic fold. Torsion has involved the whole anterior half of the embryo and is completed in the cephalic region, so that the head now lies left side down on the yolk. The posterior half of the embryo is still in its original postion, ventral surface prone on the yolk. At the extreme posterior end, the beginning of the caudal fold marks off the tail region of the embryo from the extra-embryonic membranes. The head fold of the amnion has progressed caudad, together with the lateral amniotic folds, impocketing the embryo nearly to the level of the omphalomesenteric arteries.

The cranial flexure, which was seen beginning in chicks of about 38 hours, has increased rapidly until at this stage the

brain is bent nearly double on itself. The axis of the bending being in the mid-brain region, the mesencephalon comes to be the most anteriorly located part of the head and the prosencephalon and myelencephalon lie opposite each other, ventral surface to ventral surface (Fig. 55). The original anterior end of the prosencephalon is thus brought in close proximity to the heart, and the optic vesicles and the auditory

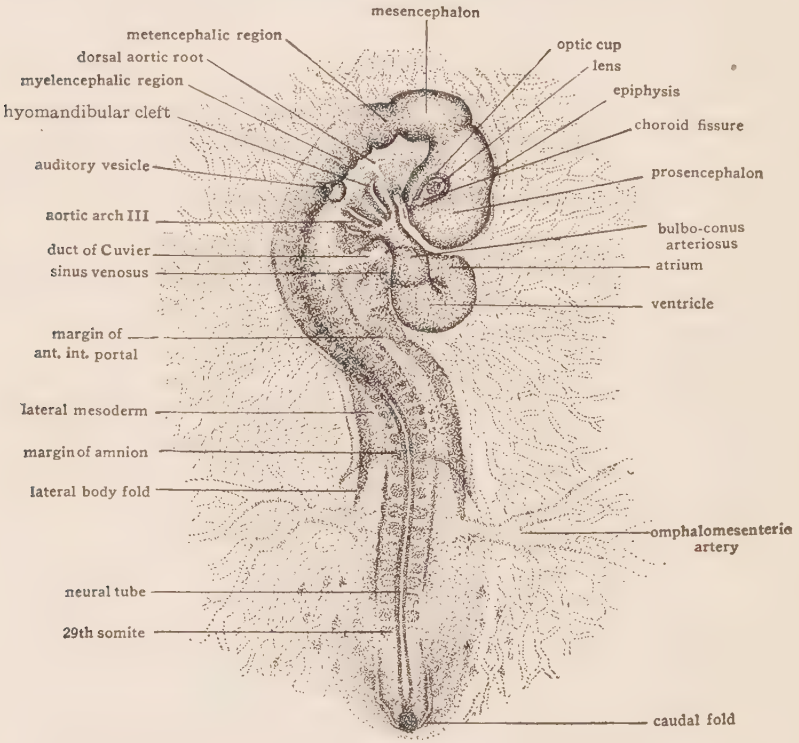


FIG. 55.—Dextro-dorsal view (× 14) of entire embryo of 29 somites (about 55 hours incubation).

vesicles are brought opposite each other at nearly the same antero-posterior level.

At this stage flexion has involved the body farther caudally as well as in the brain region. It is especially marked at about the level of the heart in the region of transition from myelencephalon to spinal cord. Since this is the future neck region of the embryo the flexure at this level is known as the cervical flexure.

II. The Nervous System

Growth of the Telencephalic Region.—The completion of torsion in the head region causes rapid changes in the configuration of the brain as seen in entire embryos between 40 and 50 hours of incubation. The same fundamental regions can, however, be identified throughout this range of development. The anterior part of the brain has undergone rapid enlargement. A slight constriction in the dorsal wall (Fig. 56) indicates the impending division of the prosencephalon into telencephalon and diencephalon (Fig. 59). Except for its considerable increase in size no important changes have taken place in the telencephalic region.

The Epiphysis.—In the mid-dorsal wall of the diencephalic region a small evagination has appeared. This evagination is the epiphysis (Figs. 55 and 56). It is destined to become differentiated into the pineal gland of the adult.

The Infundibulum and Rathke's Pocket.—In the floor of the diencephalon the infundibular depression has become deepened and lies close to a newly formed ectodermal invagination known as Rathke's pocket (Fig. 56). The epithelium of Rathke's pocket is destined to be separated from the superficial ectoderm and to become permanently associated with the infundibular portion of the diencephalon to form the hypophysis or pituitary body.

The Optic Vesicles.—The optic vesicles have undergone changes which completely alter their appearance. In 33-hour chicks they are spheroidal vesicles connected by broad stalks with the lateral walls of the prosencephalon (Fig. 39). At this stage the lumen of each optic vesicle (opticœle) is widely continuous with the lumen of the prosencephalon (prosocœle) (Fig. 46, *A*). The constriction of the optic stalk which begins to be apparent in 38-hour embryos (Fig. 40) is much more marked in 55-hour chicks.

The most striking and important advance in their development is the invagination of the distal ends of the single-walled optic vesicles to form double-walled optic cups (Fig. 57, *B*). The concavities of the cups are directed laterally. Mesially the cups are continuous, over the narrowed optic stalks, with the ventro-lateral walls of the diencephalic region of the original

prosencephalon. The invaginated layer of the optic cup is termed the sensory layer because it is destined to give rise to the sensory layer of the retina. The layer against which

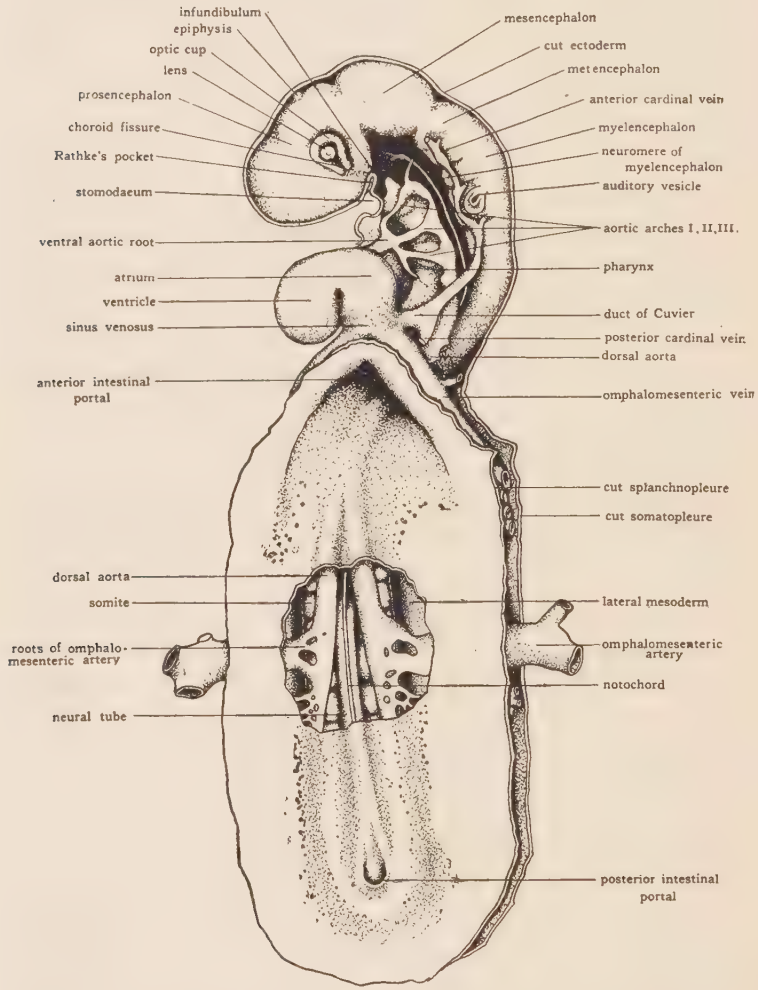


FIG. 56.—Diagram of dissection of chick of about 50 hours. (*Modified from Prentiss.*) The splanchnopleure of the yolk-sac cephalic to the anterior intestinal portal, the ectoderm of the left side of the head, and the mesoderm in the pericardial region have been dissected away. A window has been cut in the splanchopleure of the dorsal wall of the mid-gut to show the origin of the omphalomesenteric arteries.

the sensory layer comes to lie after its invagination is termed the pigment layer because it gives rise to the pigmented layer of the retina. The double-walled cups formed by invagination

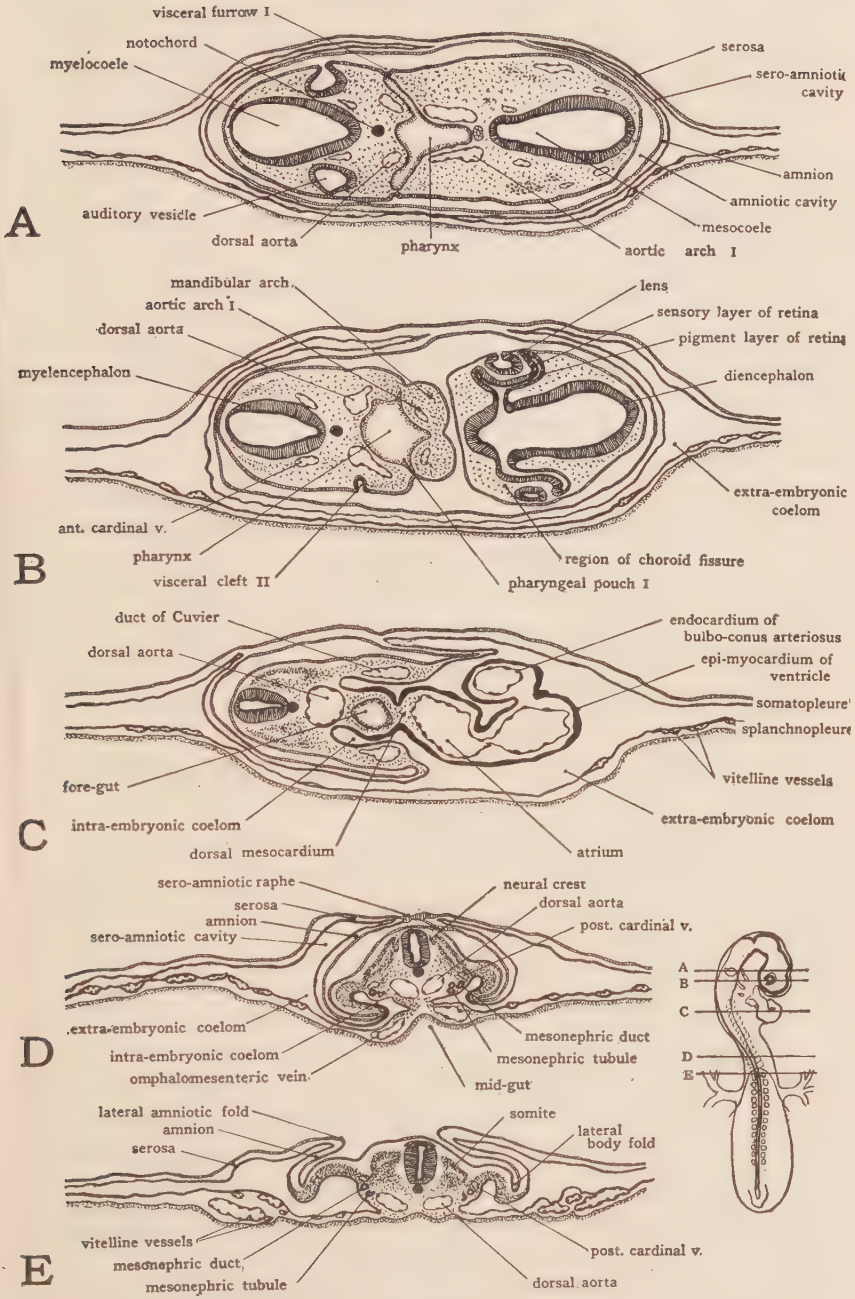


FIG. 57.—Diagrams of transverse sections of 55-hour (30-somite) chick. The location of the sections is indicated on an outline sketch of the entire embryo.

are also termed secondary optic vesicles in distinction to primary optic vesicles, as they are called before the invagination. The formerly capacious lumen of the primary optic vesicle is practically obliterated in the formation of the optic cup. What remains of the primary opticœle is now but a narrow space between the sensory and the pigment layers of the retina (Fig. 57, *B*). Later when these two layers fuse this space is entirely obliterated.

While the secondary optic vesicles are usually spoken of as the optic cups, they are not complete cups. The invagination which gives rise to the secondary optic vesicles, instead of beginning at the most lateral point in the primary optic vesicles, begins at a point somewhat toward their ventral surface and is directed mesio-dorsad. As a result the optic cups are formed without any lip on their ventral aspect. They may be likened to cups with a segment broken out of one side. This gap in the optic cup is the choroid fissure (Fig. 56). In figure 57, *B*, a section is shown which passes through the head of the embryo on a slight slant so that the right optic cup, being cut to one side of the choroid fissure, appears complete; while the left optic cup, being cut in the region of the fissure, shows no ventral lip.

The infolding process by which the optic cups are formed from the primary optic vesicles is continued to the region of the optic stalks. As a result the optic stalks are infolded so that their ventral surfaces become grooved. Later in development the optic nerves and blood vessels come to lie in the grooves thus formed in the optic stalks.

The Lens.—The lens of the eye arises independently of the optic vesicles, from the superficial ectoderm of the head. The first indications of lens formation appear in chicks of about 40 hours as local thickenings of the ectoderm immediately overlying the optic vesicles. These placodes of thickened ectoderm sink below the general level of the surface of the head to form small vesicles which extend into the secondary optic vesicles. Their opening to the surface is rapidly constricted and eventually they are disconnected altogether from the superficial ectoderm. At this stage the opening to the outside still persists although it is very small (Fig 57, *B*, right eye). In sections which do not pass directly through the opening, the lens vesi-

cle appears as if it were completely separated from the overlying ectoderm (Fig. 57, *B*, left eye).

The derivation of the lens from a placode of thickened epithelium which sinks below the general surface, and eventually loses its connection with the superficial ectoderm, is strikingly similar to the early steps in the derivation of the auditory vesicle. But these primordia, after they are separated from the ectoderm, follow divergent lines of differentiation leading to adult conditions which are structurally and functionally totally unlike. The origin of these two structures from cell groups similarly folded off from the same germ layer, but which once established undergo each their own characteristic differentiation, exemplifies a sequence of events so characteristic of developmental processes in general as to call for at least a comment in passing.

The Posterior Part of the Brain and the Cord Region of the Neural Tube.—Caudal to the diencephalon the brain shows no great change as compared with the last stages considered. The mesencephalon is somewhat enlarged and the constrictions separating it from the diencephalon cephalically and the metencephalon caudally are more sharply marked. The metencephalon is more clearly marked off from the myelencephalon and its roof is beginning to show thickening. In the myelencephalon the neuromeric constrictions are still evident in the ventral and lateral walls (Figs. 55 and 56). The dorsal wall has become much thinner than the ventral and lateral walls (Fig. 57, *A* and *B*) and shows no trace of division between the neuromeres.

In the cord region of the neural tube the lateral walls have become thickened at the expense of the lumen so that the neural canal appears slit-like in sections of embryos of this age (Fig. 57, *E*) rather than elliptical as it is immediately after the closure of the neural folds. At this stage the closure of the neural tube is completed throughout its entire length. The last regions to close were at the cephalic and caudal ends of the neural groove. In younger stages where they remained open these regions were known as the anterior neuropore and the sinus rhomboidalis, respectively.

The Neural Crest.—In the closure of the neural tube the superficial ectoderm which at first lay on either side of the

neural groove, continuous with the neural plate ectoderm, becomes fused in the mid-line and separated from the neural plate to constitute an unbroken ectodermal covering (Cf. Figs. 35, *B*, and 46, *B*). At the same time the lateral margins of the neural plate become fused to complete the neural tube. There are cells lying originally at the edges of the neural folds which

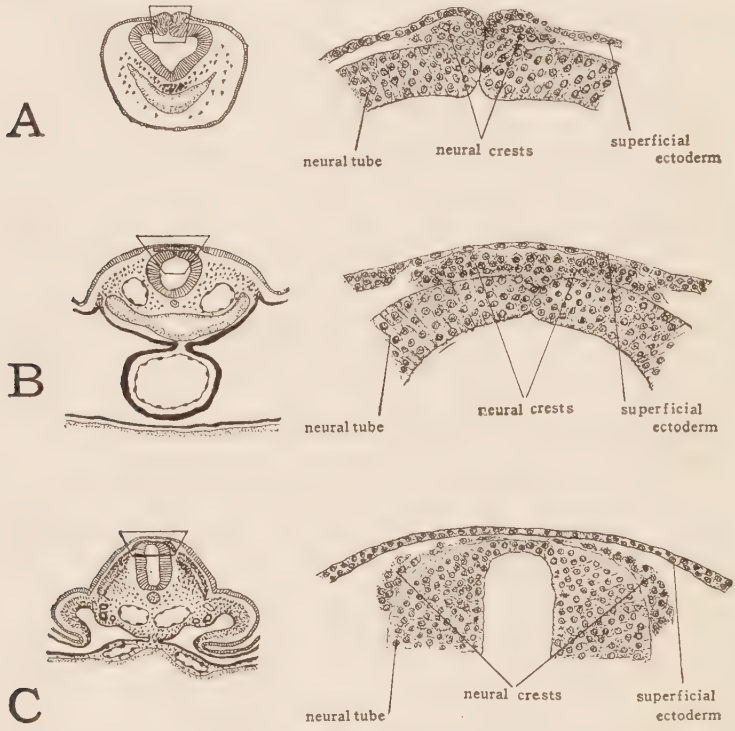


FIG. 58.—Drawings from transverse sections to show origin of neural crest cells. The location of the area drawn is indicated on the small sketch to the left of each drawing.

A, anterior rhombencephalic region of 30-hour chick; *B*, posterior rhombencephalic region of 36-hour chick; *C*, mid-dorsal region of cord in 55-hour chick.

are not involved in the fusion of either the superficial ectoderm or the neural plate. These cells form a pair of longitudinal aggregations extending one on either side of the mid-dorsal line in the angles between the superficial ectoderm and the neural tube (Fig. 58, *A*). With the fusion of the edges of the neural folds to complete the neural tube, and the fusion of the superficial ectoderm dorsal to the neural tube, these two longitudinal cell masses become for a time confluent in the mid-line

(Fig. 58, *B*). But because this aggregation of cells arises from paired components and soon again separates into right and left parts it is to be considered as potentially paired. On account of its position dorsal to the neural tube it is known as the neural crest.

The neural crest should not be confused with the margin of the neural fold with which it is associated before the closure of the neural tube. The margin of the neural fold involves cells which go into the superficial ectoderm and into the neural tube, as well as those which are concerned in the formation of the neural crest.

When first established the neural crest is continuous antero-posteriorly. As development proceeds, the cells of the neural crest migrate ventro-laterally on either side of the spinal cord (Fig. 58, *C*), and at the same time become segmentally clustered. The segmentally arranged cell groups thus derived from the neural crest give rise to the dorsal root ganglia of the spinal nerves, and in the head region to the ganglia of the sensory cranial nerves. (For a later stage of the dorsal root ganglia see figure 72.)

III. The Digestive Tract

The Fore-gut.—The manner in which the three primary regions of the gut-tract are established has already been considered in a general way (see Chapter XI and Fig. 50). In 50 to 55-hour chicks the fore-gut has acquired considerable length. It extends from the anterior intestinal portal cephalad almost to the infundibulum (Fig. 56).

As the first region of the tract to be established, the fore-gut is naturally the most advanced in differentiation. We can already recognize a pharyngeal and an œsophageal portion. The pharyngeal region lies ventral to the myelencephalon and is encircled by the aortic arches (Fig. 59). The pharynx is somewhat flattened dorso-ventrally and has a considerably larger lumen than the œsophageal part of the fore-gut (Cf. Fig. 57, *B* and *C*).

The Stomodæum.—There is at this stage no mouth opening into the pharynx. However, the location where the opening will be formed is indicated by the approximation of a ventral

outpocketing near the anterior end of the pharynx, to a depression formed in the adjacent ectoderm of the ventral surface of the head (Fig. 56). The ectodermal depression, known as the stomodæum, deepens until its floor lies in contact with the entoderm of the pharyngeal outpocketing (Fig. 56). The thin layer of tissue formed by the apposition of the stomodæal ectoderm to the pharyngeal entoderm is known as the oral plate. Later in development the oral plate breaks through bringing the stomodæum and the pharynx into open communication. Growth of surrounding structures deepens the original stomodæal depression, and it becomes the oral cavity. The region of the oral plate in the embryo becomes, in the adult, the region of transition from oral cavity to pharynx.

The Pre-oral Gut.—It will be noted by reference to figure 56 that the oral opening is not established at the extreme cephalic end of the pharynx. The part of the pharynx which extends cephalic to the mouth opening is known as the pre-oral gut. After the rupture of the oral plate, the pre-oral gut eventually disappears, but an indication of it persists for a time as a small diverticulum termed Seessell's pocket (Cf. Figs. 56 and 69).

The Mid-gut.—Although the mid-gut is still the most extensive of the three primary divisions of the digestive tract, it presents little of interest. It is nothing more than a region where the gut still lies open to the yolk. It does not have even a fixed identity. As fast as any part of the mid-gut acquires a ventral wall by the closing-in process involved in the progress of the sub-cephalic and sub-caudal folds it ceases to be mid-gut and becomes fore-gut or hind-gut. Differentiation and local specializations appear in the digestive tract only in regions which have ceased to be mid-gut.

The Hind-gut.—The hind-gut first appears in embryos of about 55 hours (Fig. 56). The method of its formation is similar to that by which the fore-gut was established. The sub-caudal fold undercuts the tail region and walls off a gut pocket. The hind-gut is lengthened at the expense of the mid-gut as the sub-caudal fold progresses cephalad and is also lengthened by its own growth caudad. It shows no local specializations until later in development.

IV. The Visceral Clefts and Visceral Arches

At this stage the chick embryo has unmistakable visceral arches and visceral clefts. Although only transitory, they are

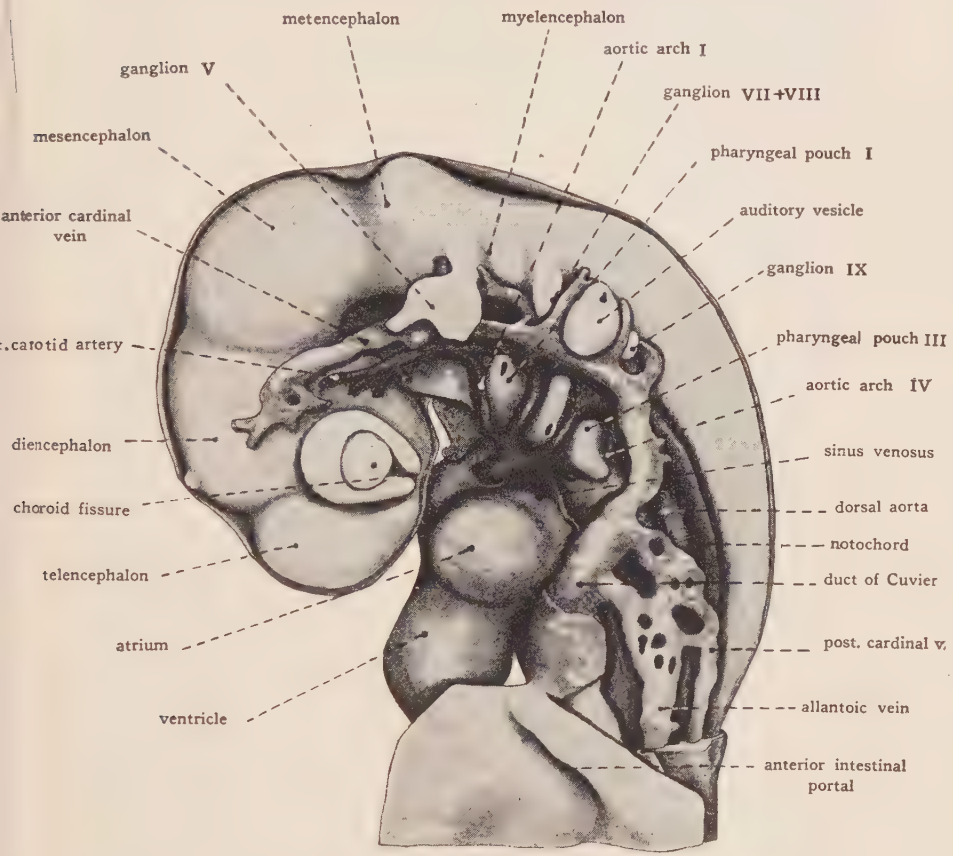


Fig. 59.—Wax plate reconstruction of cephalic and cardiac region of 60-hour chick embryo. The ectoderm and the mesenchyme of the left side have been removed to expose the underlying structures. Note especially the topography of the brain, the relations of the aortic arches to the pharyngeal pouches, and the way in which the large veins enter the sinus venosus. Though the omphalo-mesenteric veins are not fully exposed by this dissection, their position is clearly indicated by the conspicuous bulging which they cause in the ent oderm on either side of the anterior intestinal portal. Just cephalic to the portal these originally paired veins fuse with each other to enter the sinus venosus as a large median vessel.

morphologically of great importance not only from the comparative view-point, and because of their significance as structures exemplifying recapitulation, but also because of their participation in the formation of the embryonic arterial system,

of some of the ductless glands, of the eustachian tube, and of the face and jaws.

The visceral clefts are formed by the meeting of ectodermal depressions, the visceral furrows, with diverticula from the lateral walls of the pharynx, the pharyngeal pouches. During most of the time the visceral furrows are conspicuous features in entire embryos, they may be seen, by studying sections, to be closed by a thin layer of tissue composed of the ectoderm of the floor of the visceral furrow and the entoderm at the distal extremity of the pharyngeal pouch (Fig. 57, *A*). The breaking through of this thin double layer of tissue brings the pharyngeal pouches into communication with the visceral furrows thereby establishing open visceral clefts. In birds an open condition of the clefts is transitory. In the chick the most posterior of the series of clefts never becomes open. Although some of the clefts never become open and others open for a short time the term cleft is usually used to designate these structures which are potentially clefts, whether open or not.

The position of the visceral clefts is best seen in entire embryos. They are commonly designated by number beginning with the first cleft posterior to the mouth and proceeding caudad. The first post-oral cleft appears earliest in development and is discernible at about 46 hours of incubation. Visceral cleft II appears soon after, and by 50 to 55 hours three clefts have been formed (Fig. 55).

Between adjacent visceral clefts, the lateral body walls about the pharynx are thickened. Each of these lateral thickenings meets and merges in the mid-ventral line with the corresponding thickening of the opposite side of the body. Thus the pharynx is encompassed laterally and ventrally by a series of arch-like thickenings, the visceral or gill arches. The visceral arches like the visceral clefts are designated by number, beginning at the anterior end of the series. Visceral arch I lies cephalic to the first post-oral cleft, between it and the mouth region. Because of the part it plays in the formation of the mandible it is also designated as the mandibular arch. Visceral arch II is frequently termed the hyoid arch, and visceral cleft I, because of its position between the mandibular and hyoid arches, is known as the hyomandibular cleft. Posterior to the hyoid arch the

visceral arches and clefts are ordinarily designated by their post-oral numbers only.

There are other structures which are just beginning to be differentiated in the pharyngeal region and fore-gut of embryos of this stage, but it seems better to consider them in connection with later stages when their significance will be more readily grasped.

V. The Circulatory System

The Heart.—In embryos of 30 to 40 hours incubation we traced the expansion of the heart till it was bent to the right of the embryo in the form of a U-shaped tube (Figs. 37, 39, 41). The disappearance of the dorsal mesocardium except at its posterior end, leaves the mid-region of the heart lying unattached and extending to the right, into the pericardial region of the cœlom. The heart is fixed with reference to the body of the embryo at its cephalic end where the ventral aortic roots lie embedded beneath the floor of the pharynx, and caudally in the sinus region where it is attached by the omphalomesenteric veins, by the ducts of Cuvier, and by the persistent portion of the dorsal mesocardium.

During the period between 30 and 55 hours of incubation the heart itself is growing more rapidly than is the body of the embryo in the region where the heart lies. Since its cephalic and caudal ends are fixed, the unattached mid-region of the heart becomes at first U-shaped and then twisted on itself to form a loop. The atrial region of the heart is forced somewhat to the left, and the conus region is thrown across the atrial region by being twisted to the right and dorsally. The ventricular region constitutes the loop proper (Cf. Figs. 40, 47 and 55). This twisting process reverses the original cephalo-caudal relations of the atrial and ventricular regions. Before the twisting, the atrial region of the heart was caudal to the ventricular region as it is in the adult fish heart. In the twisting of the heart the atrial region, by reason of its association with the fixed sinus region of the heart, undergoes relatively little change in position. The ventricular region is carried over the dextral side of the atrium and comes to lie caudal to it, thus arriving in the relative position it occupies in the adult heart.

The bending and subsequent twisting of the heart lead toward its division into separate chambers. As yet, however, no indication of the actual partitioning of the heart is apparent. It is still essentially a tubular organ through which the blood

FIG. 60.—Dextral view of cephalic and cardiac region of chick of about 45 hours incubation. (*From Minot after Evans.*)

The blood vessels have been injected to show the capillary plexus of the head region. In the drawing the heart and arteries are differentiated from the veins and capillaries by darker shading. This figure, together with Fig. 61, shows the manner in which main vessels develop in the embryo from a primary capillary plexus. It will be noted in this figure that the part of the capillary plexus which lies more superficially already shows an enlargement in diameter and a directness of path which is indicative of the fact that it is to become the main trunk vein of this region.

passes directly, without any division into separate channels or currents.

The Aortic Arches.—In 33 to 38-hour chicks the ventral aortæ communicate with the dorsal aortæ over a single pair of aortic arches which bend around the anterior end of the pharynx (Figs. 41 and 42). With the formation of the visceral arches new aortic arches appear. The original pair of aortic arches comes to lie in the mandibular arch, and the new aortic arches are

formed caudal to the first pair, one pair in each visceral arch. In chicks of 50 to 55 hours, two pairs of aortic arches have been established, usually also the third is present, and sometimes the fourth is beginning to form (Figs. 56, 59, 60 and 61).

FIG. 61.—Dextral view of cephalic and cardiac region of injected chick of about 50 hours incubation. (*From Minot after Evans.*)

This figure shows a later stage in the development of the anterior cardinal vein from the primary capillary plexus of the head region. The main channel, but vaguely suggested in the previous figure, is here quite definite. Here also a second aortic arch has been completed and a plexiform outgrowth of vascular endothelium from the dorsal aortic root toward the ventral aorta indicates the impending formation of the third arch.

The Fusion of the Dorsal Aortæ.—The dorsal aortæ arise as vessels paired throughout their entire length (Fig. 41). As development progresses they fuse in the mid-line to form the unpaired dorsal aorta familiar in adult anatomy. This fusion takes place first at about the level of the sinus venosus and progresses thence cephalad and caudad. Cephalically it never extends to the pharyngeal region. Caudally the whole length

of the aorta is eventually involved. At this stage the fusion has progressed caudad to about the level of the 14th somite (Figs. 55, 56, and 57).

The Cardinal and Omphalomesenteric Vessels.—The relationship of the cardinal veins and the omphalomesenteric vessels are little changed from the conditions in 40 to 50-hour chicks. The posterior cardinals have elongated, keeping pace with the caudal progress of differentiation in the mesoderm. They lie just dorsal to the intermediate mesoderm in the angle formed between it and the somites (Fig. 57, *D*). The entrance of the omphalomesenteric veins into the sinus venosus, and the origin of the omphalomesenteric arteries from the dorsal aortæ show little change from conditions familiar from the study of younger embryos.

VI. The Differentiation of the Somites

When the somites are first formed they consist of nearly solid masses of cells derived from the dorsal mesoderm (Fig. 62, *A*). The cells composing them show a more or less radial arrangement. In the center of the somite a cavity is usually discernible. This cavity is at first extremely minute. In somites which have been recently formed it may be altogether wanting.

As the somite becomes more sharply marked off the radial arrangement of the outer zone of cells appears more definitely (Fig. 62, *B*). The boundaries of the central cavity are considerably extended but its lumen is almost completely filled by a core of irregularly arranged cells. In sections which pass through the middle of the somite, this central core of cells is seen to arise from the lateral wall of the somite where it is continuous with the intermediate mesoderm.

A little later in development the outer zone of cells on the ventro-mesial face of the somite loses its originally definite boundaries and becomes merged with the central core of cells. This ill-defined cell aggregation, known as the sclerotome, becomes mesenchymal in characteristics, and extends ventromesiad from the somite of either side toward the notochord (Fig. 62, *C* and *D*). The cells of the sclerotomes of either side continue to converge about the notochord and later take part in the formation of the axial skeleton.

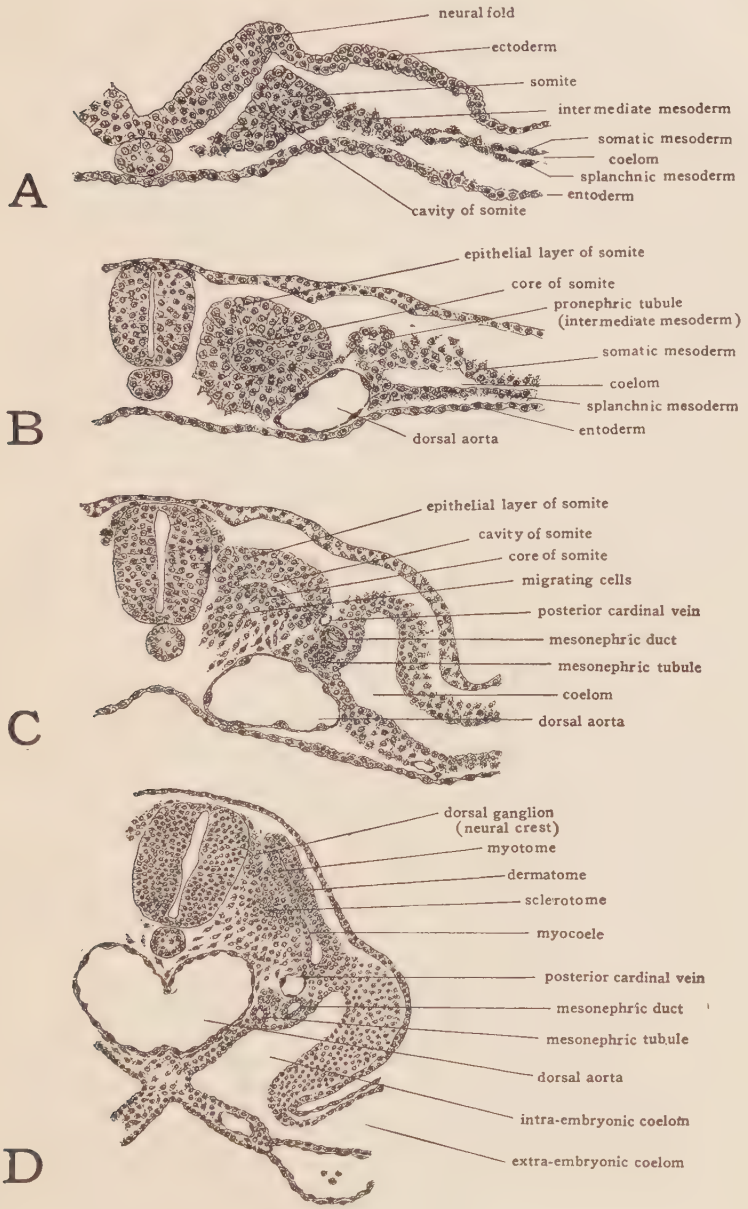


FIG. 62.—Drawings from transverse sections to show the differentiation of the somites.

A, second somite of 4-somite chick; *B*, ninth somite of 12-somite chick; *C*, twentieth somite of 30-somite chick; *D*, seventeenth somite of 33-somite chick.

During the formation of the sclerotome the dorsal part of the original outer cell-zone of the somite has maintained its definite boundaries and epithelioid characteristics. The part of this outer zone which lies parallel to the ectoderm is known as the dermatome (Fig. 62, *C* and *D*). It later becomes associated with the ectoderm and forms the deeper layers of the integument, the ectoderm giving rise to the epithelial layer only.

The dorso-mesial portion of the outer zone of the somite becomes the myotome. It is folded somewhat laterad from its original position next to the neural tube (Fig. 62, *C*) and comes to lie ventro-mesial to the dermatome and parallel to it (Fig. 62, *D*). (A later stage in the differentiation of the somite is shown in figure 72.) The portion of the original cavity which persists for a time between the dermatome and myotome is termed the myocœle. The myotomes undergo the most extensive growth of any of the parts of the somite, giving rise eventually to the entire skeletal musculature of the body, except for the muscles in the cephalic and branchial regions which are derived from head mesenchyme.

VII. The Urinary System

In the section-diagrams of figure 57, *D* and *E*, certain parts of the urinary system which have been established in chicks of 50 to 55 hours will be found located and labeled. The urinary system is relatively late in becoming differentiated. Only a few of the early steps in its formation can at this time be made out. Many structures which later become of great importance are not represented even by primordial cell aggregations. Except for those well grounded in comparative anatomy, any logical discussion of the structures which have appeared must anticipate much that occurs later in development. Consideration of the mode of origin and significance of the nephric organs appearing at this stage has, therefore, been deferred.

CHAPTER XIII

THE DEVELOPMENT OF THE CHICK DURING THE THIRD AND FOURTH DAYS OF INCUBATION

I. External Features.
   Torsion; flexion; the visceral arches and clefts; the oral region; the appendage buds; the allantois.

II. The Nervous System.
   Summary of development prior to the third day; the formation of the telencephalic vesicles; the diencephalon; the mesencephalon; the metencephalon; the myelencephalon; the ganglia of the cranial nerves; the spinal cord; the spinal nerve roots.

III. The Sense Organs.
   The eye; the ear; the olfactory organs.

IV. The Digestive and Respiratory Systems.
   Summary of development prior to the third day; the establishment of the oral opening; the pharyngeal derivatives; the trachea; the lung-buds; the œsophagus and stomach; the liver; the pancreas; the midgut region; the cloaca; the proctodæum and the cloacal membrane.

V. The Circulatory System.
   The interpretation of the embryonic circulation; the main routes of the embryonic circulation; the vitelline circulation; the allantoic circulation; the intra-embryonic circulation; the heart.

VI. The Urinary System.
   The general relationships of pronephros, mesonephros, and metanephros; the pronephric tubules of the chick: the mesonephric tubules.

VII. The Cœlom and Mesenteries.

I. External Features

Torsion.—Chicks of three days incubation (Fig. 63) have been affected by torsion throughout their entire length. Tor-

sion is complete well posterior to the level of the heart but the caudal portion of the embryo is not yet completely turned on its side. In four-day chicks the entire body has been turned through 90 degrees and the embryo lies with its left side on the yolk (Figs. 53 and 64).

Flexion.—The cranial and cervical flexures which appeared in embryos during the second day have increased so that in

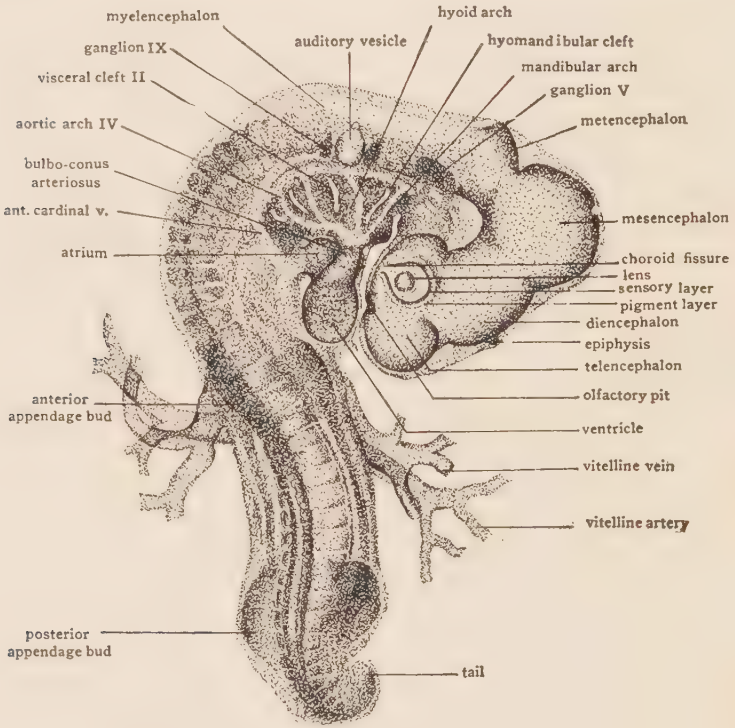


FIG. 63.—Dextro-dorsal view (× 14) of entire chick embryo of 36 somites (about three days incubation).

three-day and four-day chicks the long axis of the embryo shows nearly right-angled bends in the mid-brain and in the neck region. The mid-body region of three-day chicks is slightly concaved dorsally. This is due to the fact that the embryo is still broadly attached to the yolk in that region. By the end of the fourth day the body folds have undercut the embryo so it remains attached to the yolk only by a slender stalk. The yolk-stalk soon becomes elongated allowing the embryo to become first straight in the mid-dorsal region, and then convex

dorsally. At the same time the caudal flexure is becoming more pronounced. The progressive increase in the cranial, cervical, dorsal, and caudal flexures results in the bending of the embryo on itself so that its originally straight long-axis becomes C-shaped and its head and tail lie close together (Fig. 64).

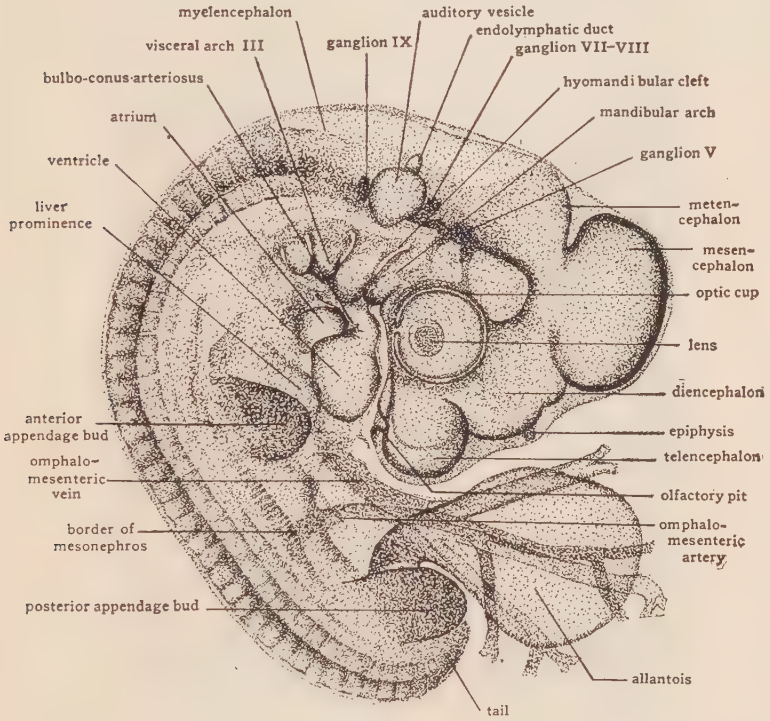


FIG. 64.—Dextral view of entire chick embryo of 41 somites (about four days incubation). Stained and cleared preparation drawn by transmitted light.

The Visceral Arches and Clefts.—A fourth visceral cleft has appeared caudal to the three that were already formed in 55-hour chicks. The visceral arches are thicker and more conspicuous than in earlier embryos. In lightly stained whole-mounts of a three-day chick it is still possible to make out the aortic arches running through the visceral arches. In a chick of four days the visceral arches have become so much thickened that it is very difficult to see the vessels traversing them.

The Oral Region.—The cervical flexure presses the pharyngeal region and the ventral surface of the head so closely to-

gether that it is difficult to make out the topography of the oral region by study of entire embryos. If the head and pharyngeal region are cut from the trunk and viewed from the ventral aspect the relations of the structures about the mouth are well shown (Fig, 67). The mandibular arch forms the caudal boundary of the oral depression. Arising on either side in connection with the mandibular arch are paired elevations, the maxillary processes, which grow mesiad and form the cephalo-

FIG. 65.—Wren embryo of an age which corresponds developmentally with a chick of 4½ days incubation. Unstained embryo photographed by reflected light to show external configuration. Compare with figure 64. This embryo and the one appearing in the following figure were from a collection made through the coöperation of the Baldwin Bird Research Laboratories.

lateral boundaries of the mouth opening. The nasal pits appear as shallow depressions in the ectoderm of the anterior part of the head which overhangs the mouth region. Surrounding each nasal pit is a U-shaped elevation with its limbs directed toward the oral cavity. The lateral limb of the elevation is the naso-lateral process, and the median limb is the naso-medial process. As development proceeds the two naso-medial processes grow toward the mouth and meet the maxillary processes which are growing in from either side. The fusion of the two naso-medial processes with each other in the mid-line,

and the fusion of each of them laterally with the maxillary process of its own side gives rise to the upper jaw (maxilla). The fusion in the mid-line of the right and left components of the mandibular arch gives rise to the lower jaw (mandible).

FIG. 66.—Wren embryo half way through its period of incubation (*i.e.* corresponding to a chick of about 10 days) microphotographed by reflected light to show external configuration. Note that by this stage of development definitely avian features have emerged from an early embryonic configuration which was much the same as that of other sauropsidan embryos (See Fig. 1). The enormous relative size of the eye and the mid-brain; the shape of the appendage buds, tail, and beak; the auditory meatus with no ear pinna; the nodules on crown, back, and tail, due to growing feather germs;—all unmistakably place the embryo as belonging to the bird class.

The Appendage Buds.—Both the anterior and posterior appendage-buds have appeared in embryos of three days. They are formed by bud-like outgrowths from somites. The anterior appendages arise opposite somites 17 to 19 inclusive, and the

posterior appendages arise opposite somites 26 to 32 inclusive. During the fourth day the appendage buds increase rapidly in size and become elongated but otherwise their appearance and their relationships show little change.

The Allantois.—The development of the extra-embryonic membranes has already been considered (Chap. XI) and needs no further discussion here. In order to show the embryos more clearly, the extra-embryonic membranes, except for the allan-

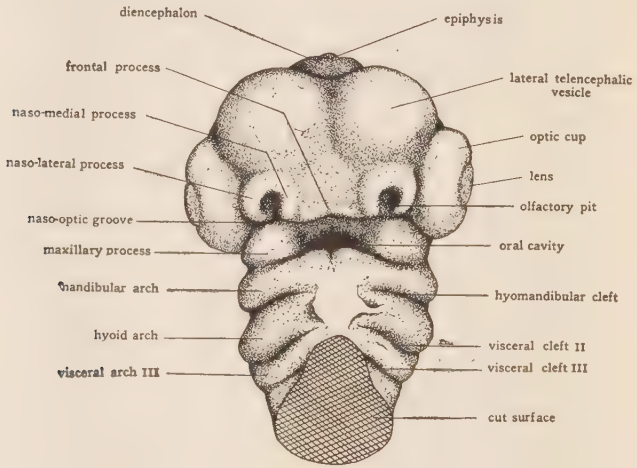


FIG. 67.—Drawing to show the external appearance of the structures in the oral region of a four-day chick. Ventral aspect.

tois, have been removed from the specimens drawn in figures 63 and 64. The cut edge of the amnion shows at its anterior attachment to the body, opposite the anterior appendage bud and just caudal to the tip of the ventricle. The allantois in the three-day chick is as yet small and is concealed by the posterior appendage buds. In four-day embryos it has undergone rapid enlargement and projects from the umbilical region as a stalked vesicle of considerable size.

II. THE NERVOUS SYSTEM

Summary of Development Prior to the Third Day.—The earliest indication of the formation of the central nervous system appears in chicks of 16 to 18 hours as a local thickening of the ectoderm which forms the neural plate (Fig. 27). The neural plate then becomes longitudinally folded to form the neural groove (Figs. 31, 32, 33). By fusion of the margins of

the neural folds, first in the cephalic region and later caudally, the neural groove is closed to form a tube and at the same time separated from the body ectoderm. The cephalic portion of the neural tube becomes dilated to form the brain and the remainder of the neural tube gives rise to the spinal cord (Figs. 36 and 39).

In its early stages the brain shows a series of enlargements in its ventral and lateral walls, indicative of its fundamental metameric structure. In the establishment of the three-vesicle condition of the brain, the lines of demarcation between prosencephalon, mesencephalon, and rhombencephalon are formed by the exaggeration of certain of the inter-neuromeric constrictions and the obliteration of others (see Chap. IX and Fig. 38). The original neuromeric enlargements persist longest in the rhombencephalon.

The three-vesicle condition of the brain is transitory. By forty hours the division of the rhombencephalon into metencephalon and myelencephalon is clearly indicated (Figs. 38, *D* and 40). The division of the prosencephalon and the establishment of the five-vesicle condition characteristic of the adult brain, does not take place until somewhat later.

In chicks of 55 hours (Figs. 55 and 56) the appearance of the cranial flexure has resulted in the bending of the brain so that the entire prosencephalon is displaced ventrad and then toward the heart. At the same time the head of the embryo has undergone torsion and lies with its left side on the yolk. Although flexion and torsion have thus completely changed the general appearance of the brain as seen in entire embryos, the regions already established in 40-hour chicks are still evident. The prosencephalon has, however, become very noticeably enlarged cephalic to the optic vesicles, and a slight constriction in its dorsal wall indicates the beginning of the demarcation of the telencephalic region from the diencephalic region.

The Formation of the Telencephalic Vesicles.—By the end of the third day the antero-lateral walls of the primary forebrain have been evaginated to form a pair of vesicles lying one on either side of the mid-line (Figs. 63, 67, and 68, *B*). These lateral evaginations are known as the telencephalic vesicles. The openings through which their cavities are continuous with the lumen of the median portion of the brain are later known

as the foramina of Monro. The telencephalic division of the brain includes not only the two lateral vesicles but also the median portion of the brain from which they arise. The telocœle has therefore three divisions, a median, broadly con-

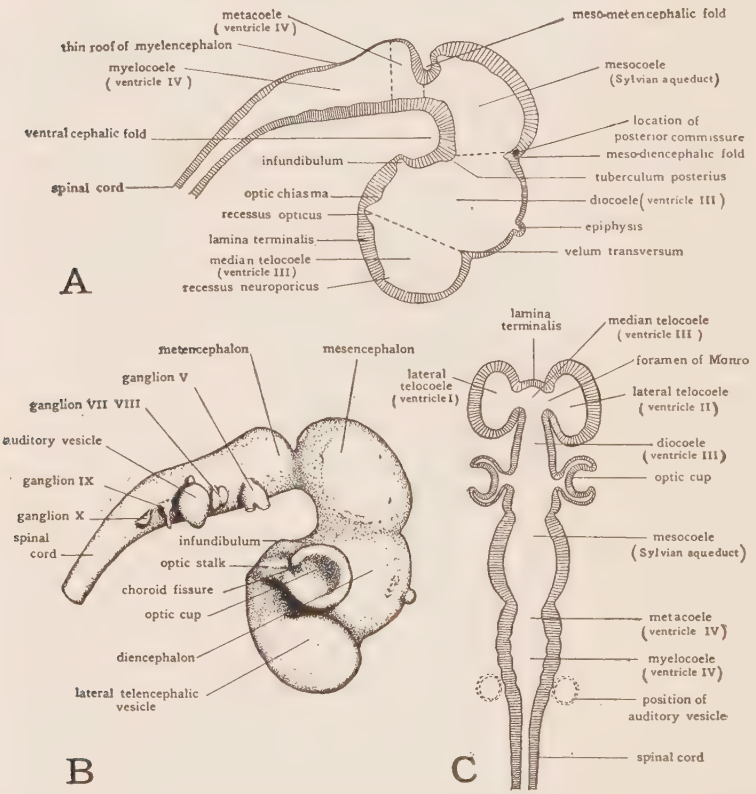


FIG. 68.—Diagrams to show the topography of the brain of a four-day chick. *A*, plan of sagittal section. The arbitrary boundaries between the various brain vesicles (according to von Kupffer) are indicated by broken lines. *B*, dextral view of a brain which has been dissected free. *C*, schematic frontal section plan of brain. The flexures of the brain are supposed to have been straightened before the section was cut.

fluent posteriorly with the diocœle, and two lateral, connecting with the median through the foramina of Monro (Fig. 68, *C*).

Before the formation of the telencephalic vesicles the most anterior part of the brain lay in the mid-line, but the rapid growth of the telencephalic vesicles soon carries them anteriorly beyond the median portion of the telocœle. The median anterior wall of the telocœle which formerly was the most anterior

part of the brain, and which remains the most anterior part of the brain lying in the mid-line, is known as the lamina terminalis (Figs. 68, *A*, and *C*, and 69). The telencephalic vesicles become the cerebral hemispheres, and their cavities become the paired lateral ventricles of the adult brain. The hemispheres undergo enormous enlargement in their later development and extend dorsally and posteriorly as well as anteriorly, eventually covering the entire diencephalon and mesencephalon under their posterior lobes. They contain the brain-centers for memory and for all actions conditioned by past experience.

As a matter of convenience in dealing with the morphology of the brain, more or less arbitrary lines of division between the adjacent brain regions are recognized. The division between telencephalon and diencephalon is an imaginary line drawn from the velum transversum to the recessus opticus (Fig. 68, *A*). Velum transversum is the name given to the internal ridge formed by the deepening of the dorsal constriction which was first noted in chicks of 55 hours as indicating the impending division of the primary fore-brain (Fig. 56). The recessus opticus is a transverse furrow in the floor of the brain which in the embryo leads on either side into the lumina of the optic stalks.

The Diencephalon.—The lateral walls of the diencephalon at this stage show little differentiation except ventrally where the optic stalks merge into the walls of the brain. The development of the epiphysis as a median evagination in the roof of the diencephalon has already been mentioned (Chap. XII). Except for some elongation it does not differ from its condition when first formed in embryos of about 55 hours. The infundibular depression in the floor of the diencephalon has become appreciably deepened and lies in close proximity to Rathke's pocket with which it is destined to fuse in the formation of the hypophysis (Fig. 69). Later in development the lateral walls of the diencephalon become greatly thickened to form the thalami, thus reducing the size and changing the shape of the diocœle, which is known in adult anatomy as the third brain ventricle. The anterior part of the roof of the diencephalon remains thin and becomes richly vascular. Later these vessels, invaginating the roof with them, push into the third ventricle to form the anterior choroid plexus.

The boundary between the diencephalon and the mesencephalon is an imaginary line drawn from the internal ridge formed by the original dorsal constriction between the primary fore-brain and mid-brain, to the tuberculum posterius (Fig. 68, *A*). The tuberculum posterius is a rounded elevation in the floor of the brain, of importance chiefly because it is regarded

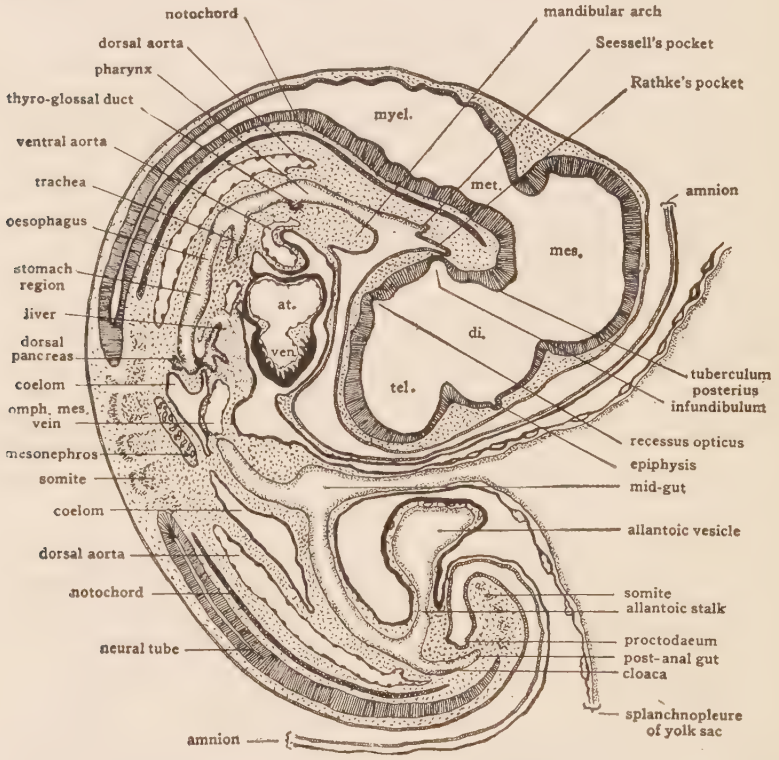


FIG. 69.—Diagram of median longitudinal section of four-day chick. Due to a slight bend in the embryo the section is para-sagittal in the mid-dorsal region but for the most part it passes through the embryo in the sagittal plane.

as marking the boundary between diecephalon and mesencephalon.

The Mesencephalon.—The mesencephalon as yet shows no specializations, beyond a thickening of its walls. The dorsal walls of the mesencephalon later increase rapidly in thickness and become the corpora quadrigemina of the adult brain. As the name implies these are four symmetrically placed elevations. The anterior pair constitute the brain-center for vision, the

posterior pair are the center for hearing. The floor of the mesencephalon also becomes greatly thickened and is known in the adult as the crura cerebri. It serves as the main pathway of the fiber tracts which connect the cerebral hemispheres with the posterior part of the brain and the spinal cord. The originally capacious mesocœle is thus reduced by the thickening of the walls about it to a narrow canal (Aqueduct of Sylvius).

The Metencephalon.—The boundary between the mesencephalon and metencephalon is indicated by the original interneuromeric constriction which separated them at the time of their establishment (Cf. Figs. 38 and 68). The caudal boundary of the metencephalon is not definitely defined. It is regarded as being located approximately at the point where the brain roof changes from the thickened condition characteristic of the metencephalon to the thin condition characteristic of the myelencephalon. The metencephalon shows practically no differentiation in four-day chicks. Later there is ventrally and laterally an extensive ingrowth of fiber tracts giving rise to the pons and to the cerebellar peduncles. The roof of the metencephalon undergoes extensive enlargement and becomes the cerebellum of the adult brain, the coördinating center for complex muscular movements.

The Myelencephalon.—The dorsal myelencephalic wall is reduced in thickness, indicative of its final fate as the thin roof of the medulla. It later receives a rich supply of small blood vessels which, carrying the roof with them, grow into the myelocœle to form the posterior choroid plexus. The ventral and lateral myelencephalic walls become the floor and sidewalls of the medulla. Functionally the medulla serves both as a conduction path between cord and brain, and as a reflex center for involuntary activities such as breathing.

The Ganglia of the Cranial Nerves.—In the brain region, cells derived from the cephalic portion of the neural crest have become aggregated to form ganglia. The largest and the most clearly defined of the ganglia present in four-day chicks is the Gasserian ganglion of the fifth (trigeminal) cranial nerve (Fig. 70). It lies ventro-laterally, opposite the most anterior neuromere of the myelencephalon. From its cells sensory nerve fibers grow mesiad into the brain and distad to the face and mouth region. In four-day chicks the beginning of the

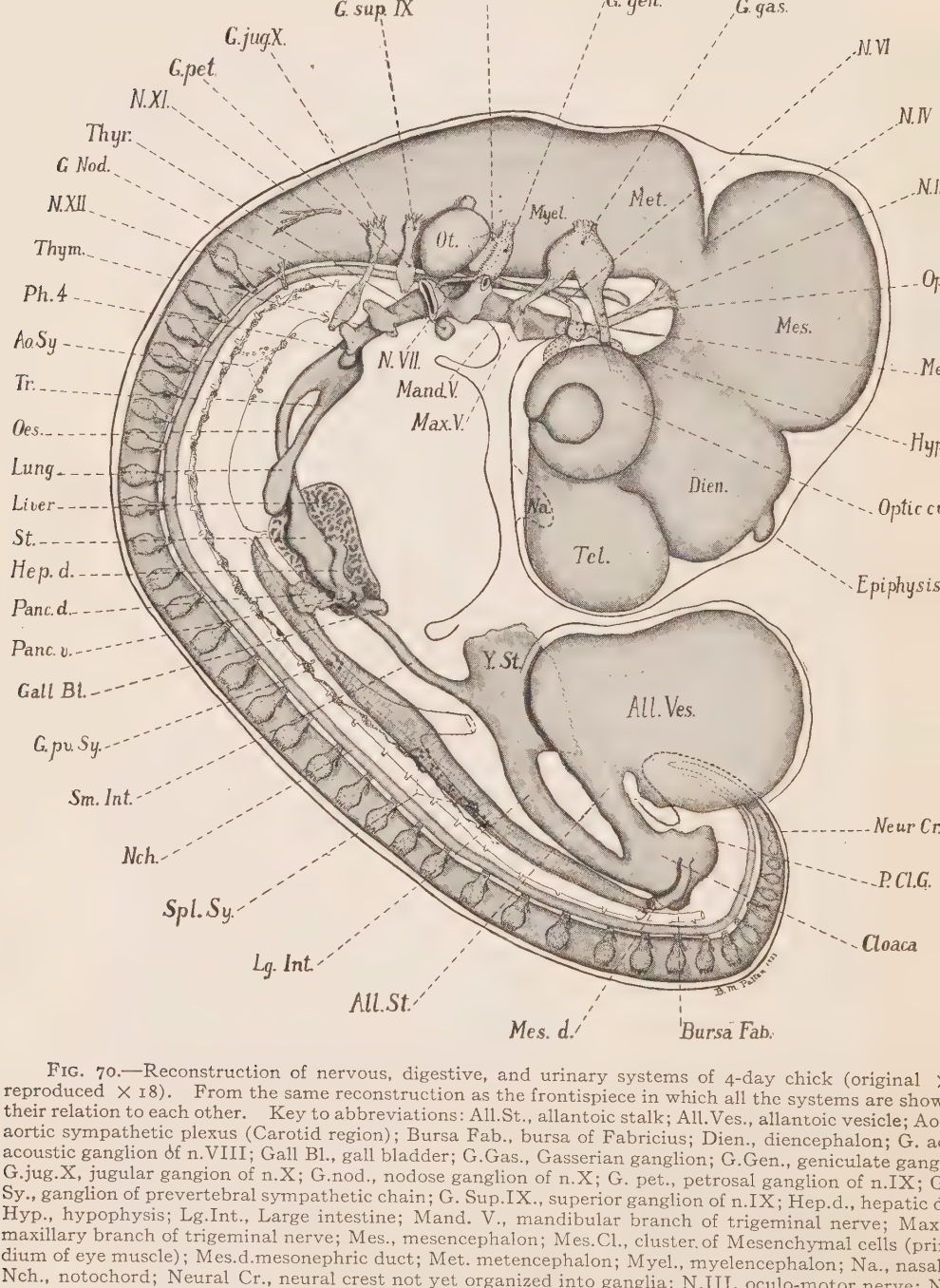


FIG. 70.—Reconstruction of nervous, digestive, and urinary systems of 4-day chick (original × reproduced × 18). From the same reconstruction as the frontispiece in which all the systems are show their relation to each other. Key to abbreviations: All.St., allantoic stalk; All.Ves., allantoic vesicle; Ao aortic sympathetic plexus (Carotid region); Bursa Fab., bursa of Fabricius; Dien., diencephalon; G. a acoustic ganglion of n.VIII; Gall Bl., gall bladder; G.Gas., Gasserian ganglion; G.Gen., geniculate gang G.jug.X, jugular gangion of n.X; G.nod., nodose ganglion of n.X; G. pet., petrosal ganglion of n.IX; G Sy., ganglion of prevertebral sympathetic chain; G. Sup.IX., superior ganglion of n.IX; Hep.d., hepatic d Hyp., hypophysis; Lg.Int., Large intestine; Mand. V., mandibular branch of trigeminal nerve; Max maxillary branch of trigeminal nerve; Mes., mesencephalon; Mes.Cl., cluster of Mesenchymal cells (pri dium of eye muscle); Mes.d.mesonephric duct; Met. metencephalon; Myel., myelencephalon; Na., nasal Nch., notochord; Neural Cr., neural crest not yet organized into ganglia; N.III, oculo-motor nerve; N trochlear nerve; N.VI, abducens nerve; N.VII, facial nerve; N.XI, Spinal Accessory nerve; N.XII, H glossal nerve; Oes., oesophagus; Oph.V., ophthalmic branch of trigeminal nerve; Ot., otocyst (audi vesicle); Panc.d., dorsal pancreas; Panc.v., ventral pancreas; P.Cl.g., postcloacal gut; Ph.4, fourth pha geal pouch; Sm.Int., small intestine; Spl.Sy., aortic sympathetic plexus (splanchnic region); St., stom Thym., thymic diverticulum of 3rd pharyngeal pouch; Thyr., median thyroid diverticulum; Tel., t cephalon; Tr., trachea; Y.St., yolk stalk.

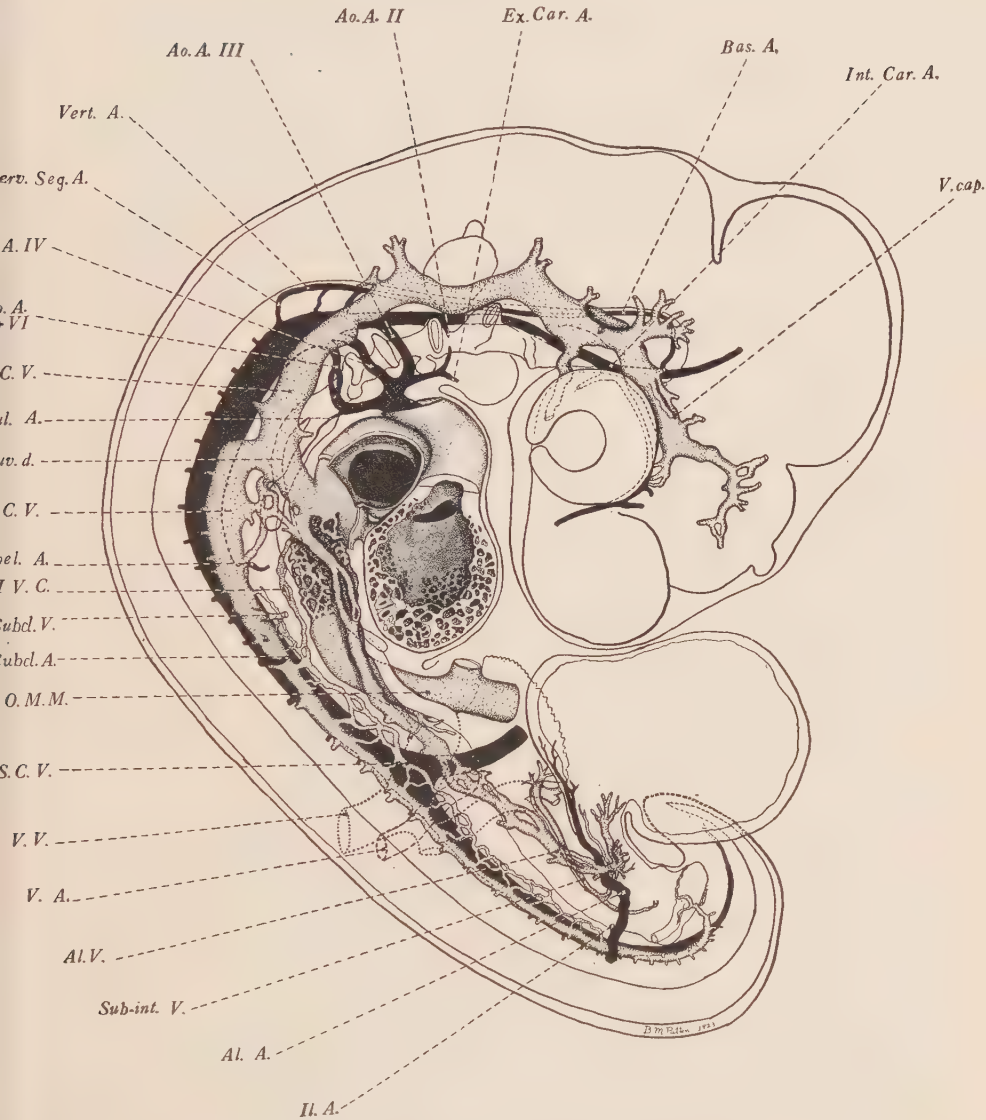


FIG. 71.—Reconstruction of circulatory system of 4-day chick (original × 51, reproduced × 18) m the same reconstruction as the frontispiece and Fig. 70. These three illustrations should be studied ether. Key to abbreviations: A.C.V., anterior cardinal vein; Al.A., allantoic artery; Al.V., allantoic n; Ao.A.II, aortic arch II (disappearing); Ao.A.III, aortic arch III (carotid arch); Ao.A.IV, aortic arch Ao.A.V & VI, aortic arch VI with aortic arch V appearing as a loop attached to VI; Bas.A., basilar ery; Cerv.Seg.A., cervical segmental artery; Coel.A., coeliac artery; Cuv.d., duct of Cuvier; Ex.Car.A., ernal carotid artery; Il.A., iliac artery; Int.Car.A., internal carotid artery; I.V.C., posterior vena cava; P.-., posterior cardinal vein; Pul.A., pulmonary artery; S.C.V., sub-cardinal vein; Subcl.A., subclavian ery; Subcl.V., subclavian vein; Sub.int.V., sub-intestinal vein; V.A., vitelline artery; V.cap., vena capitis; t.A., vertebral artery; V.O.M.M., omphalomesenteric veins at their confluence to form an unpaired dian vessel; V.V., vitelline vein.

ophthalmic division of the fifth nerve extends from the ganglion toward the eye, and the beginning of the mandibulo-maxillary division is growing toward the angle of the mouth (Fig. 70). Immediately cephalic to the auditory vesicle is a mass of neural crest cells which is the primordium of the ganglia of the seventh and eighth nerves. The separation of this double primordium to form the geniculate ganglion of the seventh nerve and the acoustic ganglion of the eighth nerve begins during the fourth day. Posterior to the auditory vesicle the ganglion of the ninth nerve can be clearly seen even in whole-mounts (Fig. 64). The ganglia of the tenth (vagus) nerves can be recognized in sections of chicks at the end of the fourth day but are difficult to make out in whole-mounts.

The Spinal Cord.—The spinal cord region of the neural tube when first established exhibits a lumen which is elliptical in cross section. As development progresses the lateral walls of the cord become greatly thickened in contrast with the dorsal and ventral walls which remain thin. In this process the lumen (central canal) becomes compressed laterally until it appears in cross section as little more than a vertical slit. The thin dorsal wall of the tube is known as the roof plate; the thin ventral wall, as the floor plate; and the thickened side walls are called the lateral plates.

The Spinal Nerve Roots.—During the fourth day the establishment of the spinal nerve roots has begun. The growth of nerve fibers from the neuroblasts can be traced only with the aid of special methods of staining. The more general steps in the development of the roots of the spinal nerves can, however, be followed in sections prepared by the ordinary methods.

In the adult each spinal nerve is connected with the cord by two roots, a dorsal root which is sensory in function, and a ventral root which is motor in function. Lateral to the cord the dorsal and ventral roots unite. The spinal ganglion (dorsal root ganglion) is located on the dorsal root between the spinal cord and the point where dorsal and ventral roots unite. Distal to the union of dorsal and ventral roots is a branch, the ramus communicans, which extends ventrad to a ganglion of the sympathetic nerve cord.

When first formed from the neural crest cells, the spinal ganglion has no connection with the cord (Fig. 58). The dorsal

root is established by the growth of nerve fibers from cells of the spinal ganglion mesiad into the dorsal part of the lateral plate of the cord. At the same time fibers grow distad from these cells to form the peripheral part of the nerve (Fig. 72). The fibers which arise from the dorsal root ganglion conduct sensory impulses toward the cord.

Coincident with the establishment of the dorsal root, the ventral root is formed by fibers which grow out from cells located in the ventral part of the lateral plate of the cord

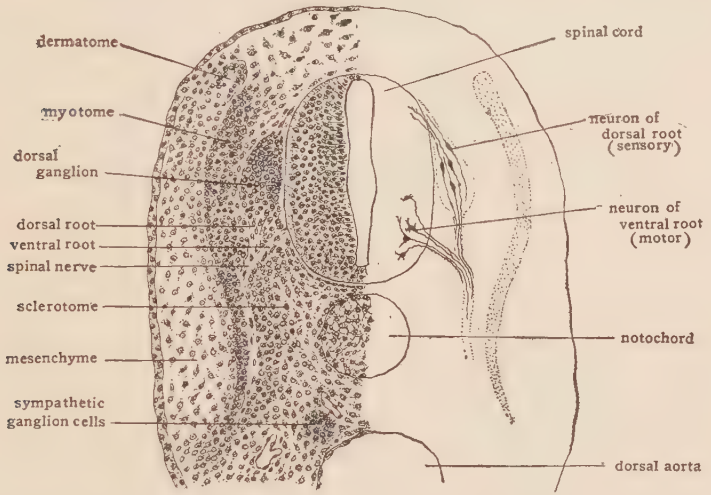


FIG. 72.—Drawing to show the structure and relations of a spinal ganglion and the roots of a spinal nerve. The left half of the drawing represents structures as they appear after treatment by the usual nuclear staining method. The right half of the section shows schematically the nerve cells and the fibers growing out from them as they may be demonstrated by the Golgi method. (*Nerve cells and fibers after Ramon y Cajál.*)

(Fig. 72). The fibers which thus arise from cells in the cord and pass out through the ventral root, conduct motor impulses from the brain and cord to the muscles with which they are associated peripherally.

The sympathetic ganglia arise from cells of the neural crest, and of the spinal cord, which migrate ventrally and form masses lying on either side of the mid-line at the level of the dorsal aorta (Fig. 72). By the end of the fourth day these cells constitute a pair of cords in which enlargements can be made out opposite the spinal ganglia (Fig. 70). These enlargements are the primary sympathetic ganglia. Each sympathetic ganglion

is connected with the corresponding spinal nerve by a cellular cord which is the primordium of the ramus communicans. Later, both sensory and motor fibers appear in the rami communicantes, putting the sympathetic ganglia in communication with the spinal nerve roots. From certain of the sympathetic ganglia, cells migrate still farther ventrad, establishing the primordia of the splanchnic sympathetic system. (Fig. 70.)

III. The Sense Organs

The Eye.—The primary optic vesicles arise in chicks of about 30 hours as dilations in the lateral wall of the prosencephalon (Figs. 37 and 41). At first the optic vesicles open broadly into the brain, but later constrictions develop which narrow their attachment to the form of a stalk (Fig. 40). In chicks of 55 hours the primary optic vesicles are invaginated to form the double-walled secondary optic vesicles or optic cups. The invagination takes place in such a way that the ventral wall of the cup is incomplete, the gap in it being known as the choroid fissure (Figs. 57, *B*, and 59).

The lens arises as a thickening of the superficial ectoderm which becomes depressed to form a vesicular invagination extending into the optic cup (Fig. 57, *B*).

In chicks of four days the choroid fissure has become narrowed by the growth of the walls of the optic cup on either side of it (Fig. 68, *B*). At the same time the orifice of the optic cup becomes narrowed by convergence of its margins toward the lens (Fig. 73, *A*). Meanwhile the lens has become freed from the superficial ectoderm and forms a completely closed vesicle. Sections of the lens at this stage show that the cells constituting that part of its wall which lies toward the center of the optic cup are becoming elongated to form the lens fibers (Fig. 73, *C*).

At this stage we can identify the beginning of most of the structures of the adult eye. The thickened internal layer of the optic cup will give rise to the sensory layer of the retina (Fig. 73, *B*). Fibers arise from nerve cells in the retina and grow along the groove in the ventral surface of the optic stalk toward the brain to form the optic nerve. The external layer of the optic cup gives rise to the pigment layer of the retina. Mesenchymal cells can be seen aggregating about the outside of the optic cup. From these the sclera and choroid coat are

derived. Some of the mesenchyme makes its way into the optic cup through the choroid fissure and gives rise to the cellular elements of the vitreous body. The complex ciliary appara-

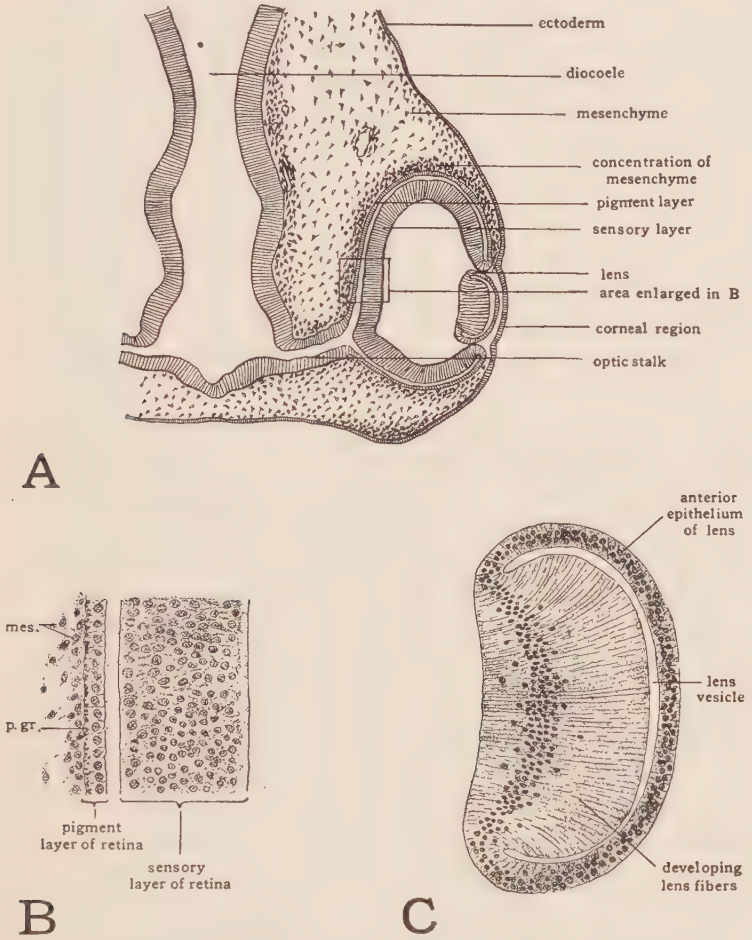


FIG. 73.—Drawings to show structure of the eye of a four-day chick. *A*, diagram to show topography of eye region; *B*, drawing to show cellular organization of the pigment and sensory layers of the retina. Abbreviations: mes., mesenchymal cell; p.gr., pigment granule; *C*, drawing to show cellular organization of the lens.

tus of the adult eye is derived from the margins of the optic cup adjacent to the lens. The corneal and conjunctival epithelium arise from the superficial ectoderm overlying the eye. Mesenchymal cells which make their way between the lens and

the corneal epithelium give rise to the substantia propria of the cornea.

The Ear.—Of the structures taking part in the formation of the ear, the first to appear is the auditory placode. The auditory placode is recognizable in 36-hour chicks as a thickened plate of ectoderm. Almost as soon as it appears the placode sinks below the level of the surrounding ectoderm to form the floor of the auditory pit (Fig. 40). By constriction of its opening to the surface, the epithelium of the auditory pit becomes separated from the ectoderm of the head and comes to lie close to the lateral wall of the myelencephalon (Fig. 57, *A*). A tubular stalk, the endolymphatic duct, remains for a time adherent to the superficial ectoderm, marking the location of the original invagination (Fig. 64).

The degree of development reached by the ear primordium in four-day chicks gives little indication of the nature of the later processes by which the ear is formed. The auditory vesicle by a very complex series of changes will give rise to the entire epithelial portion of the internal ear mechanism. Nerve fibers arising from the acoustic ganglion grow into the brain proximally and to the internal ear distally establishing nerve connections between them. There is at this stage no indication of the differentiation of the external auditory meatus. The dorsal and inner portion of the hyomandibular cleft which gives rise to the eustachian tube and to the middle ear chamber has not yet become associated with the auditory vesicle.

The Olfactory Organs.—The olfactory organs are represented in three-day and four-day chicks by a pair of depressions in the ectoderm of the head. These so-called olfactory pits are located ventral to the telencephalic vesicles and just anterior to the mouth (Figs. 64 and 67). By growth of the processes which surround them, the olfactory pits become greatly deepened. The epithelium lining the pits eventually comes to lie at the extreme upper part of the nasal chambers and constitutes the olfactory epithelium. Nerve fibers grow from these cells to the telencephalic lobes of the brain to form the olfactory nerves.

IV. The Digestive and Respiratory Systems

Summary of Development Prior to the Third Day.—The primary entoderm which gives rise to the epithelial lining of the

digestive and respiratory systems and their associated glands (Fig. 26) becomes established as a separate layer before the egg is laid. In its early relationships the entoderm is a sheet-like layer of cells lying between the ectoderm and the yolk and attached peripherally to the yolk (Fig. 22). The primitive gut is the cavity bounded dorsally by the entoderm and ventrally by the yolk (Fig. 50, *A*).

Only the part of the entoderm which lies within the embryonal area is involved in the formation of the enteric tract. The peripheral portion of the entoderm goes into the formation of the yolk-sac. There is at first no definite line of demarcation between the entoderm destined to be incorporated into the body of the embryo and that which remains extra-embryonic in its associations. The foldings which appear later separating the body of the embryo from the yolk, establish for the first time the boundaries between intra-embryonic and extra-embryonic entoderm (Figs. 49 and 52).

The first part of the gut to acquire a complete entodermic lining is the fore-gut. Its floor is formed by the caudally progressing concrescence of the entoderm which takes place as the sub-cephalic and lateral body folds undercut the cephalic part of the embryo (Figs. 2, 3, and 50, *B*). At a considerably later stage the hind-gut is formed in a similar manner by the progress of the sub-caudal fold toward the head (Figs. 56 and 50, *C*). Between the fore-gut and the hind-gut, the mid-gut remains open to the yolk ventrally. As the embryo is more completely separated from the yolk, the fore-gut and hind-gut increase in extent at the expense of the mid-gut. By the fourth day of incubation the mid-gut is reduced to the region where the yolk stalk opens into the enteric tract (Figs. 50, *D* and 69).

The Establishment of the Oral Opening.—When first established the gut ends as a blind pocket both cephalically and caudally. The mouth opening does not appear until the third day, the cloacal opening is not established until much later in incubation. In embryos of 55 hours the processes leading toward the establishment of the oral opening are clearly indicated. A mid-ventral evagination of the pharynx is established immediately cephalic to the mandibular arch (Fig. 56). Opposite this outpocketing of the pharynx, and growing in to meet it, the

stomodæal depression is formed. The thin membrane formed by the meeting of the pharyngeal entoderm with the stomodæal ectoderm is known as the oral plate. The communication of the fore-gut with the outside is finally established by the breaking through of the oral plate.

The formation of the oral opening in the manner described does not take place at the extreme anterior end of the fore-gut. A small gut pocket extends cephalic to the mouth. This so-called pre-oral gut rapidly becomes less conspicuous after the breaking through of the oral plate. The small depression which in older embryos marks its location is known as Seessell's pocket (Fig. 69). Even this small depression eventually disappears altogether. Its importance lies wholly in the fact that it indicates for some time the place at which ectoderm and entoderm originally became continuous in the formation of the oral opening.

The Pharyngeal Derivatives.—Several structures arise in the pharyngeal region which do not become parts of the digestive system. Nevertheless the origin of their epithelial portions from fore-gut entoderm and their early association with this part of the gut tract make it convenient to take them up in connection with the digestive system.

The thyroid gland arises from the floor of the pharynx as a median diverticulum which makes its appearance at the level of the second pair of pharyngeal pouches. Toward the end of the fourth day the thyroid evagination has become saccular and retains its connection with the pharynx only by a narrow opening at the root of the tongue known as the thyro-glossal duct (Figs. 69 and 70). The paired evaginations which arise posteriorly from the fourth pharyngeal pouches are known as post-branchial bodies (Fig. 70). Their significance is not altogether clear. Homologous evaginations in mammals have been said to contribute to the formation of the thyroid. In the chick the post-branchial bodies do not appear to form thyroid tissue. Moreover, recent work on these structures in mammals indicates that in that group also, they probably do not give rise to thyroid tissue as formerly believed, in spite of the very close positional relation which comes to exist between them and the median thyroid evagination.

The thymus of the chick is barely indicated, if present at all, on the fourth day of incubation. It takes its origin primarily from diverticula arising from the posterior faces of the third and fourth pharyngeal pouches. The original epithelial character of the thymus is soon largely lost in an extensive ingrowth of mesenchyme and the organ becomes chiefly lymphoid in its histological characteristics.

The Trachea.—The first indication of the formation of the respiratory system appears in 3-day chicks as a mid-ventral groove in the pharynx. Beginning just posterior to the level of the fourth pharyngeal pouches and extending caudad, (Fig. 74, *B*) this laryngo-tracheal groove deepens rapidly and by closure of its dorsal margins becomes separated from the pharynx except at its cephalic (laryngeal) end. The tube thus formed is the trachea, and the opening which persists between the laryngeal end of the trachea and the pharynx is the glottis (Fig. 69). The original entodermal evagination gives rise only to the epithelial lining of the trachea, the supporting structures of the tracheal walls being derived from the surrounding mesenchyme.

The Lung-buds.—The tracheal evagination grows caudad and bifurcates to form a pair of lung-buds. As the lung-buds develop they grow into the loose mesenchyme on either side of the mid-line. The adjacent splanchnic mesoderm is pushed ahead of them in their caudo-lateral growth and comes to constitute the outer investment of the lung-buds. The entodermal buds give rise only to the epithelial lining of the bronchi, and the air passages and air chambers of the lungs. The connective tissue stroma of the lungs is derived from mesenchyme surrounding the lung-buds, and their pleural covering from the investment of splanchnic mesoderm (Fig. 75, *D*).

The Oesophagus and Stomach.—Immediately caudal to the glottis is a narrowed region of the fore-gut which becomes the œsophagus, and farther caudally a slightly dilated region which becomes the stomach (Fig. 70). The concentration of mesenchymal cells about the entoderm of the œsophageal and gastric regions foreshadows the formation of their muscular and connective tissue coats (Fig. 75, *C*, *E*).

The Liver.—In all vertebrates the liver arises as a diverticulum from the ventral wall of the gut immediately caudal to the

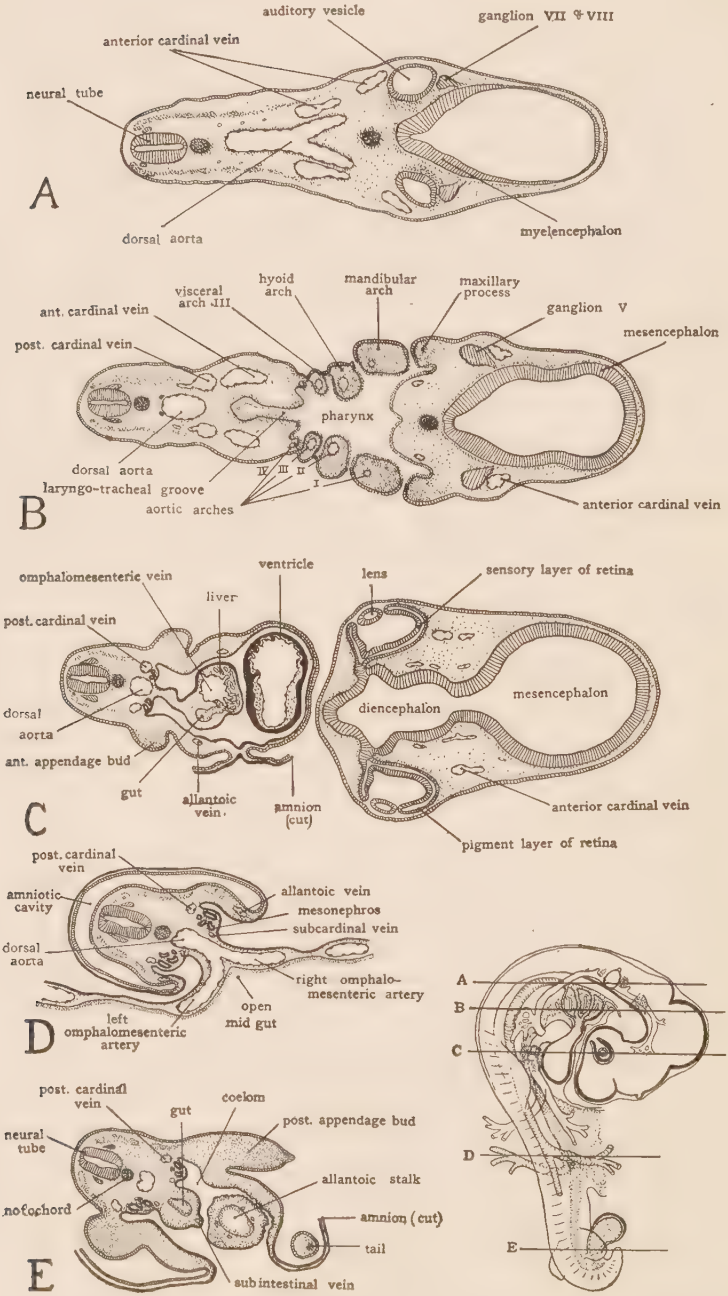


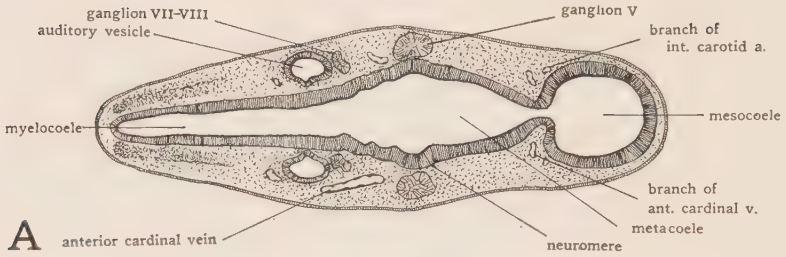
FIG. 74.—Diagrams of transverse sections of a three-day chick. The location of the sections is indicated on the small outline sketch of the entire embryo.

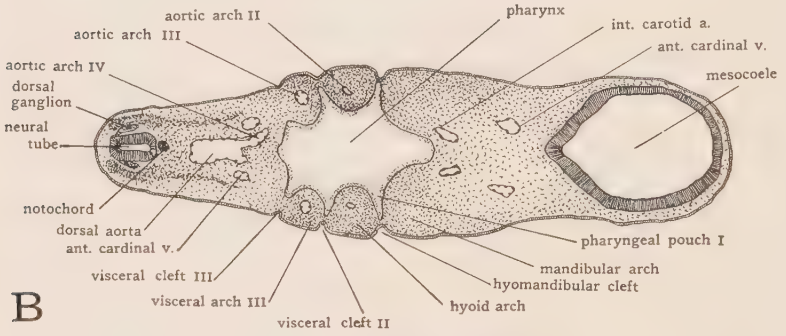
stomach region. In chick embryos the liver diverticulum appears just as the part of the gut from which it arises is acquiring a floor by the concrescence of the margins of the anterior intestinal portal. As a result the liver evagination appears for a short time on the lip of the intestinal portal, and grows cephalad toward the fork where the omphalomesenteric veins enter the sinus venosus. As closure of the gut floor is completed, the liver diverticulum comes to lie in its characteristic position in the ventral wall of the gut. In embryos of four days the original evagination has grown out in the form of branching cords of cells and become quite extensive in mass (Fig. 70). In its growth the liver pushes ahead of it the splanchnic mesoderm which surrounds the gut, with the result that the liver from its first appearance is invested by mesoderm. (Figs. 69, 74, *C*, and 75, *E*.)

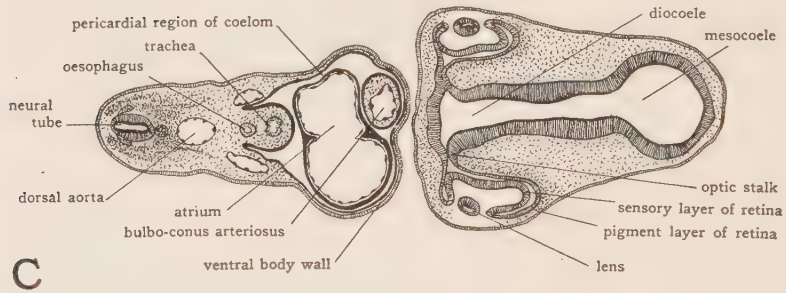
The proximal portion of the original evagination remains open to the intestine, and serves as the duct of the liver. This primitive duct later undergoes regional differentiation and gives rise in the adult to the common bile duct, to the hepatic and cystic ducts, and to the gall bladder. The cellular cords which bud off from the diverticulum become the secretory units of the liver (hepatic tubules).

The same process of concrescence which closes the floor of the fore-gut involves the proximal portion of the omphalomesenteric veins which, when they first appear, lie in the lateral folds of the anterior intestinal portal (Fig. 56). As the intestinal portal moves caudad in the lengthening of the fore-gut, the proximal portions of the omphalomesenteric veins are brought together in the mid-line and become fused. The fusion extends caudad nearly to the level of the yolk-stalk (Fig. 71). Beyond this point they retain their original paired condition. In its growth the liver surrounds the fused portion of the omphalomesenteric veins (Frontispiece, Figs. 74, *C*, and 75, *E*). This early association of the omphalomesenteric veins with the liver fore-shadows the way in which the proximal part of the afferent vitelline circulation is to be involved in the establishment of the hepatic-portal circulation of the adult.

The Pancreas.—The pancreas is derived from evaginations appearing in the walls of the intestine at the same level as the liver diverticulum. There are three pancreatic buds, a median


ganglion VII–VIII
auditory vesicle
ganglion V
branch of
int. carotid a.
mesocoele
myelocoele
branch of
ant. cardinal v.
metacoele
A
anterior cardinal vein
neuromere


aortic arch II
aortic arch III
pharynx
int. carotid a.
ant. cardinal v.
aortic arch IV
dorsal
ganglion
mesocoele
neural
tube
notochord
dorsal aorta
ant. cardinal v.
pharyngeal pouch I
mandibular arch
hyomandibular cleft
visceral cleft III
visceral arch III
hyoid arch
B
visceral cleft II


pericardial region of coelom
diocoele
trachea
mesocoele
oesophagus
neural
tube
optic stalk
sensory layer of retina
dorsal aorta
atrium
pigment layer of retina
bulbo-conus arteriosus
ventral body wall
C
lens

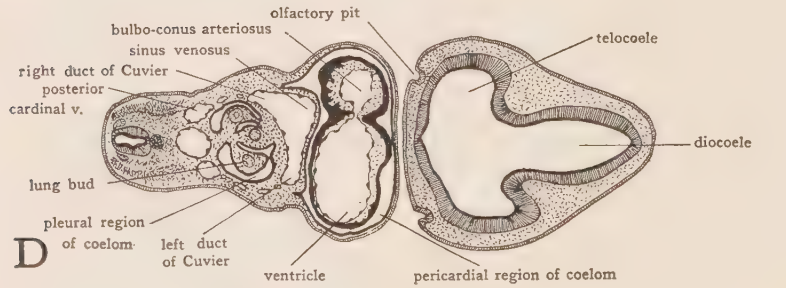

olfactory pit
bulbo-conus arteriosus
telocoele
sinus venosus
right duct of Cuvier
posterior
cardinal v.
diocoele
lung bud
pleural region
of coelom
left duct
of Cuvier
D
ventricle
pericardial region of coelom

FIG. 75.

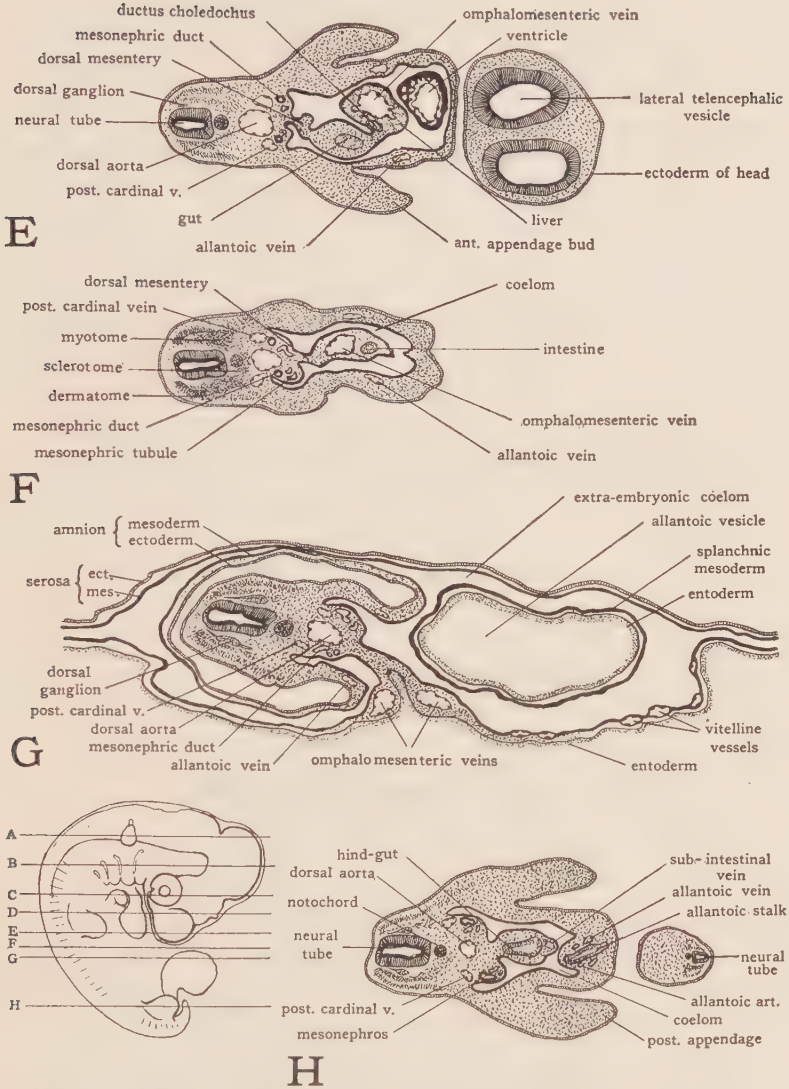


FIG. 75.—Diagrams of transverse sections of a four-day chick. The location of the sections is indicated on a small outline sketch of the entire embryo.

dorsal, and a pair of ventro-lateral buds. The dorsal evagination appears at about 72 hours, the ventro-lateral evaginations toward the end of the fourth day. The dorsal pancreatic bud arises directly opposite the liver diverticulum (Fig. 70) and grows into the dorsal mesentery (Fig. 87). The ventro-lateral buds arise where the duct of the liver connects with the intestine so that the ducts of the liver and the ventral pancreatic ducts open into the intestine by a common duct (ductus choledochus). Later in development the masses of cellular cords derived from the three pancreatic primordia grow together and become fused into a single glandular mass, but, in birds, usually two and in rare cases all three of the original ducts persist in the adult.

The Mid-gut Region.—In chicks of four days the enteric tract shows no local differentiation from the level of the liver to the cloaca except where the yolk-sac is attached. All of the gut tract between the stomach and the yolk-stalk, and the anterior third of the gut lying caudal to the yolk-stalk is destined to become the small intestine. The posterior two-thirds of the hind-gut becomes large intestine and cloaca.

The Cloaca.—The beginning of the formation of the cloaca is indicated in chicks of four days incubation, by a dilation of the posterior portion of the hind-gut (Fig. 70). Although extensive differentiation in the cloacal region does not appear until later in development, certain of its fundamental relationships are established at this stage.

The cloaca of an adult bird is the common chamber into which the intestinal contents, the urine, and the products of the reproductive organs are received for discharge. The first appearance of the cloaca in the embryo as a dilated terminal portion of the gut establishes at the outset the relations of cloaca and intestine familiar in the adult.

Although the urinary system is not at this stage developed to conditions which resemble those in the adult the parts of it which have been established are already definitely associated with the cloaca. The proximal portion of the allantoic stalk, which is the homologue of the urinary bladder of mammals, opens directly into the cloaca (Fig. 70). When the urinary system of the embryo is considered, we shall see that the ducts which drain the developing excretory organs also open into the cloacal region on either side of the allantoic stalk.

There is at this stage but little indication of the formation of the gonads. The relation of the sexual ducts to the cloaca can be made out only by the study of older embryos.

The Proctodæum and the Cloacal Membrane.—Indications of the formation of the cloacal opening to the outside appear during the fourth day of incubation. Its establishment is accomplished in much the same manner as the establishment of the oral opening. A ventral out-pocketing of the hind-gut arises just caudal to the point at which the allantoic stalk opens into the cloaca (Fig. 70). At the same time a depression appears in the overlying ectoderm. The external depression which grows in toward the gut pocket is known as the proctodæum. The double epithelial layer formed by the meeting of gut entoderm with proctodæal ectoderm is the cloacal membrane. The formation of the proctodæum and the cloacal membrane clearly indicate the location of the future cloacal opening although an open communication is not established by the rupture of the cloacal membrane until considerably later. The cloacal opening does not form at the extreme posterior end of the hind-gut and there is, therefore, a post-anal pocket of the hind-gut suggestive of the pre-oral pocket of the fore-gut.

V. The Circulatory System

The Interpretation of the Embryonic Circulation.—The embryonic circulation is difficult to understand only when the meaning of its arrangement is overlooked. If one bears in mind the significance of the circulatory system in organic economics, and the fact that any embryo must go through certain ancestral phases of organization before it can arrive at its adult structure, the changes in the arrangement of vascular channels during the course of development form a coherent and logical story.

In the embryo as in the adult the main vascular channels lead to and from the centers of metabolic activity. The circulating blood carries food from the organs concerned with its absorption, to parts of the body remote from the source of supplies; oxygen to all the tissues of the body, from organs which are especially adapted to facilitate the taking of oxygen

into the blood; and waste materials, from the places of their liberation, to the organs through which they are eliminated. One of the primary reasons the arrangement of the vessels in an embryonic bird or mammal differs so much from that in the adult, is the fact that the embryo lives under conditions totally unlike those which surround its parents. Its centers of metabolic activity are, therefore, different; and, since the course of its main blood vessels is determined by these centers, the vascular plan is different. No such profound changes as we find in birds and mammals occur between the embryonic and the adult stages in the circulation of a fish where embryo and adult are both living under similar conditions.

The organs which in the adult carry out such functions as digestion and absorption, respiration, and excretion are extremely complex and highly differentiated. They are for this reason slow to attain their definitive condition and are not ready to become functional until toward the close of the embryonic period. Moreover the conditions which surround certain of the developing organs during embryonic life would prevent their becoming functional even were they sufficiently developed so to do. Suppose the lungs, for example, were functionally competent at an early stage of development. The fact that the embryo is re-living ancestral conditions submerged in the amniotic fluid renders its lungs as incapable of functioning as those of a man under water. Likewise the developing stomach of a chick encased in its shell can receive no raw food materials by way of the mouth. Further examples are not necessary to make it obvious that were the embryo dependent on the same organs which carry on metabolism in the adult, development would be at an impasse.

Nevertheless an embryo must solve the problem of existence during the time in which it is building up a set of organs similar to those of its parents. The chick must have not only the raw food material supplied by its mother in the form of yolk, it must have in addition a means of digesting the yolk, absorbing it, and carrying it to the places where it can be utilized. Furthermore the utilization of food material to produce the energy expressed in growth depends on the presence of oxygen. So for growth there must be a means of securing oxygen and carrying it, as well as food, to all parts of the body. Nor can continued

growth go on unless the waste products liberated by the growing tissues are eliminated. At the outset of its development the embryo must, therefore, establish organs for the digestion and absorption of food, the securing of oxygen, and the elimination of waste products. In various types of embryos these functions are carried out by the yolk-sac or the allantois or by both together, in a manner depending on the exigencies of the embryo's living conditions. These structures are very primitive in comparison with the organs which carry out the corresponding functions in the adult, but their activities are so essential and so extensive that the vascular channels supplying them are a dominating feature in the embryonic circulation.

While main circulatory channels are thus always established in relation to centers of metabolic activity, we find in many situations in the embryo an unmistakable phylogenetic impress on the manner of their establishment or on the detail of their arrangement. Take, for example, the aortic arches. The blood leaving the ventrally located heart must pass around the gut to reach the dorsally located aorta. In fishes six pairs of aortic arches encircle the pharynx, breaking up in the gills into capillaries which carry out the indispensable function of oxygenating the blood. Once an animal has replaced its gills with lungs it makes little difference functionally whether or not the blood passes by each gill cleft on its way from heart to aorta. In adult birds and mammals we find this communication simplified to a single main aortic arch. But in the embryos of both birds and mammals the whole series of symmetrical aortic arches appear for a time, encircling the pharynx and passing in close relation to vestigial gill clefts. This can be interpreted only as a recapitulation of ancestral conditions,—conditions which although they have ceased to be of functional importance, appear nevertheless as a developmental phase on the way to a more highly differentiated plan of adult structure. Our interpretation of the aortic arches, then, would take into consideration first the fundamental functional necessity of a channel connecting the ventrally located heart with the dorsally located aorta. Secondly, looking at the striking arrangement of the series of aortic arches in relation to the gill arches and clefts, one sees a repetition of the structural plan which existed in water-living ancestral forms. Thus in the end we are led

back again to functional significance; for the relationship of the aortic arches to the gill arches writes into the story of individual development an unequivocal record of the evolutionary phase when the gills were a center of primary metabolic importance.

Applied to the development of the circulatory system as a whole, this tendency to repeat phyletic history means that the earliest form in which the circulatory mechanism of a bird or a mammal appears is patterned after a more primitive type and, therefore, can not be a miniature of adult conditions. The simple tubular heart pumping blood out over aortic arches to be distributed over the body by the aorta and returned to the posterior part of the heart by a bilaterally symmetrical venous system, in short the foundational vascular plan which we see in sauropsidan embryos, is essentially the plan of the circulation in fishes. When we realize this, we are puzzled neither by the early appearance of a full complement of aortic arches nor by their subsequent disappearance to make way for a new respiratory circulation in the lungs. Starting from a logical beginning in simpler ancestral conditions, we see the march of progress toward the consummation of embryonic life with the attainment of an organization like that of the immediate parent. And at each turning point the direction of further advance is sharply limited. Development can proceed only along lines that permit the maintenance of an efficient metabolism both in the temporary organs characteristic of embryonic life, and in the slowly developing organs which must be prepared to meet adult living conditions.

Finally, we must bear in mind that only relatively late in development, as the various organs become established in their adult relationships, can we expect to see the emergence of the vascular plan characteristic of the adult. Following the primitive embryonic stage there must be transitional phases when functioning embryonic channels are present side by side with channels being prepared to take over their activity in the adult. For all changes in the circulatory system of a living organism must be gradual. Any changes which were sufficiently abrupt to interfere with the circulation would result in disaster for the embryo. Even slight curtailment of the normal blood supply to any region would cause its growth

to cease; any marked local decrease in the circulation would result in local atrophy or malformation; complete interruption of any important circulatory channel, even for a short time, would inevitably mean the death of the embryo.

The Main Routes of the Embryonic Circulation.—Before taking up the various parts of the circulatory system in detail it might be well to consider briefly the main routes of the embryonic circulation. The circulation of young chick embryos involves three main arcs of which the heart is the common center and pumping station. One of these circulatory arcs, the vitelline, carries blood to the yolk-sac where food materials are absorbed and then returns the food-laden blood to the heart for distribution within the embryo. Another arc carries blood to and from the allantois. The distal portion of the allantois lies close beneath the egg shell and the blood circulating in the allantoic vessels is thereby brought into a location where interchange of gases can be carried on with the air which penetrates the shell (Fig. 49, *C* and *D*). It is in the allantoic circulation that the blood gives off its carbon dioxide and acquires a fresh supply of oxygen. The allantoic circulation is also the embryo's means of eliminating nitrogenous waste material from the blood. The remaining circulatory arc is confined to the body of the embryo. The intra-embryonic circulation has many distributing and collecting vessels, but all of them are alike in function in that they bring food material and oxygen to, carry waste material from, the various parts of the developing body. Nowhere in their course are the vessels of the intra-embryonic circulation involved in adding food material or oxygen to that already contained in the blood they convey, and nowhere do they free the blood from waste materials until well along in development, when the nephroi become functional.

In the heart the blood from the three circulatory arcs is mingled. As it leaves the heart the mixed blood is not as rich in food material as the blood coming in through the omphalomesenteric veins, nor as free from waste materials and as rich in oxygen as the blood returned over the allantoic veins.[1]

[1] There is a tendency among students who have done but little work on the circulatory system to regard any vessel which carries oxygenated blood as an artery, and any vessel which carries blood poor in oxygen and high in carbon dioxide content as a vein. This is not entirely correct even for the circulation of adult mammals on which the conception is based. In comparative anatomy

Its condition of serviceability to the embryo is, however, constantly maintained at a good average by the incoming vitelline and allantoic blood.

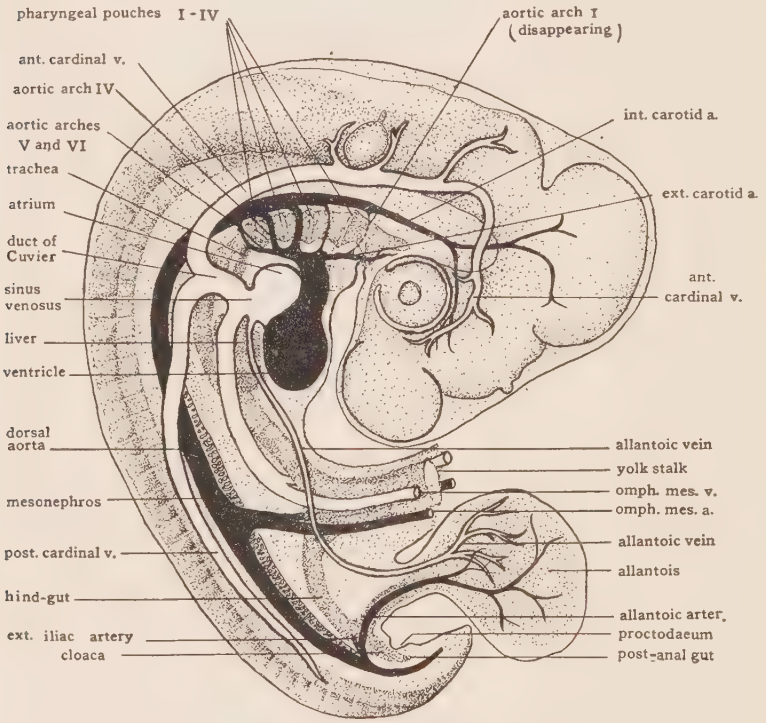


FIG. 76.—Simplified plan showing the location and relations of the main vascular channels of the four-day chick. Except for the omphalomesenteric arteries and veins paired structures are represented only on the side toward the observer. Compare with Frontispiece and Fig. 71.

The Vitelline Circulation.—The earliest indication of blood and blood vessel formation is at the chick's source of food

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and especially in embryology it is far from being the case. It is necessary, therefore, in dealing with the circulation of the embryo to eradicate this is not uncommon misconception.

The differentiation between arteries and veins which holds good for all forms, both embryonic and adult, is based on the structure of their walls, and on the direction of their blood flow with reference to the heart. An artery is a vessel carrying blood away from the heart under a relatively high, fluctuating pressure due to the pumping of the heart. Correlated with the pressure conditions in it, its walls are heavily reinforced by elastic tissue and smooth muscle. A vein is a vessel carrying blood toward the heart under a relatively low and constant pressure from the blood welling into it from capillaries. Correlated with such pressure conditions, the walls of a vein have much less smooth muscle than artery walls, and their connective tissue is predominantly of the non-elastic type.

supply. Blood islands appear in the extra-embryonic splanchnopleure of the yolk-sac toward the end of the first day of incubation, and rapidly become differentiated to form vascular endothelium enclosing central clusters of primitive blood cor-

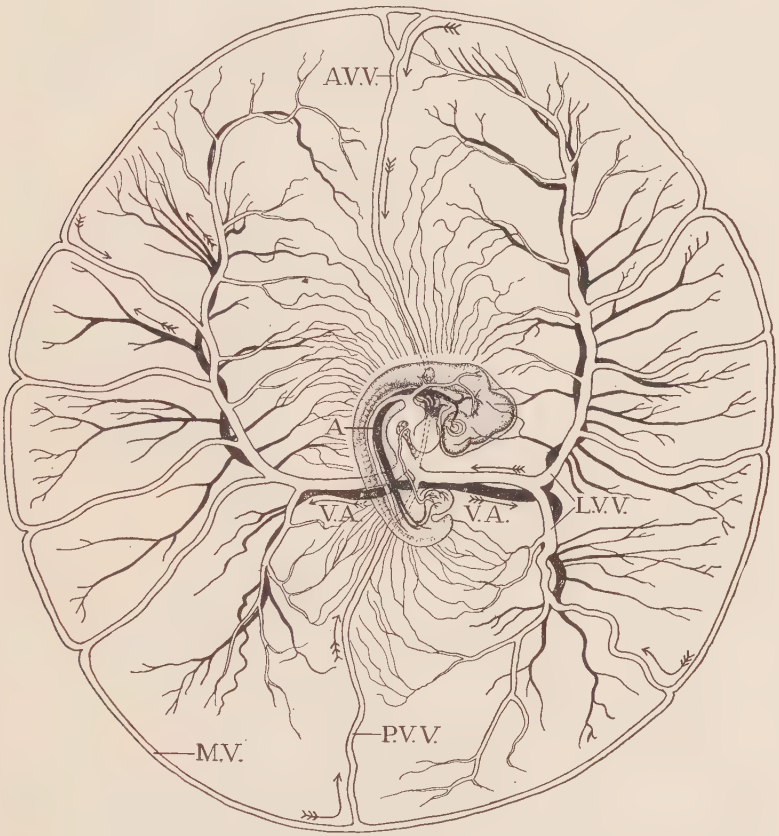


FIG. 77.—Diagram to show course of vitelline circulation in chick of about four days. (*After Lillie.*) For the intra-embryonic vessels see Fig. 71. Abbreviations; A, dorsal aorta; A.V.V., anterior vitelline vein; L.V.V., lateral vitelline vein; M.V., marginal vein (sinus terminalis); P.V.V., posterior vitelline vein; V.A., vitelline artery. The direction of blood flow is indicated by arrows.

puscles (Fig. 43). By extension and anastomosing of neighboring islands a plexus of blood channels is formed in the yolk-sac. Further extension of the vitelline plexus brings it into communication with the omphalomesenteric veins which have been developed in the embryo as caudal extensions of the heart (Figs. 39 and 45).

Toward the end of the second day of development the omphalomesenteric arteries establish communication between the dorsal aortæ and the vitelline plexus. (See Chap. X and Figs. 47 and 48.) There is now a system of open channels leading from the embryo to the yolk-sac, and back again to the embryo. With the completion of these channels the heart begins to pulsate, circulation of the blood is thereby established, and the blood cells formed in the yolk-sac are for the first time carried into the body of the embryo.

The course of the vitelline circulation in chicks of four days is shown diagrammatically in figures 76 and 77. Circulating in the rich plexus of small vessels on the yolk, the blood finally makes its way either directly into one of the larger vitelline veins, or into the sinus terminalis which acts as a collecting channel, and then through the sinus terminalis to one of the vitelline veins. The vitelline veins converge toward the yolk-stalk where they empty into the omphalomesenteric veins. The omphalomesenteric veins, at first paired throughout their entire length, have been brought together proximally by the closure of the ventral body wall and become fused to form a median vessel within the body of the embryo. It is through this vessel that the vitelline blood eventually reaches the heart. In the heart the blood of the vitelline, intra-embryonic, and allantoic circulations is mingled. The mixed blood passes out by the ventral aorta and the aortic arches into the dorsal aorta. Leaving the dorsal aorta through the vitelline arteries the blood is returned to the yolk-sac.

It should not be inferred that the blood stream "picks up" deutoplasmic granules and carries them to the embryo. The acquisition of food material by the blood depends on the activities of the entodermal cells lining the yolk-sac. These cells secrete digestive enzymes which break down the deutoplasmic granules. The liquified material is then absorbed by the yolk-sac cells and transferred to the blood. The blood carries the food material in soluble form to the embryo where it is finally assimilated.

The Allantoic Circulation.—The allantoic arteries arise by the prolongation and enlargement of the segmental vessels arising from the aorta at the level of the allantoic stalk. Their size increases rapidly as the allantois increases in extent. From

them the blood is distributed in a rich plexus of vessels which ramify in the mesoderm of the allantois (Figs. 71 and 76).

The situation of the allantois directly beneath the porous shell is such that the blood can carry on interchange of gases with the outside air (Fig. 49, *D*). It is in the rich plexus of small allantoic vessels where the surface exposure is very great that the blood gives off its carbon dioxide and takes up oxygen.

At a later stage of development the ducts of the embryonic excretory organs open into the allantoic stalk near its cloacal end. As the excretory organs become functional, the allantoic vesicle becomes the repository for the nitrogenous waste materials eliminated through them. The watery portion of the waste materials is passed off by evaporation. The remaining solids are deposited in the allantoic vesicle. They accumulate in the extra-embryonic portion of the allantois and there remain until that portion of the allantois is discarded at the close of embryonic life.

The blood from the allantois is collected and returned to the heart over the allantoic veins. From the distal portion of the allantois the smaller veins converge and unite into two main vessels, right and left, which enter the body of the embryo with the allantoic stalk (Fig. 75, *H*). After their entrance into the body the allantoic veins extend cephalad in the lateral body walls (Figs. 71 and 75, *H* to *E*). They enter the sinus venosus on either side of the entrance of the omphalomesenteric vein.

The Intra-embryonic Circulation.—The earliest vessels of the intra-embryonic circulation to appear are the large vessels communicating with the heart. In chicks of 33 hours the ventral aorta leads off from the heart cephalically and bifurcates ventral to the pharynx giving rise to a single pair of aortic arches. The aortic arches pass dorsad around the antero-lateral walls of the pharynx and are continued caudally along the dorsal wall of the gut as the paired dorsal aortæ (Figs. 41 and 42).

When, toward the end of the second day of incubation, visceral clefts and visceral arches appear, the original pair of aortic arches comes to lie in the mandibular arch. In each of the visceral arches posterior to the mandibular, new aortic arches are formed connecting the ventral aortæ with the dorsal

aortæ. By 55 hours three pairs of aortic arches are present and a fourth is beginning to form (Figs. 56 and 59).

At about this stage the dorsal aortic roots are prolonged anteriorly. The vessels thus established extend cephalad in close association with the brain as the internal carotid arteries. At a later stage the ventral aortic roots also, are extended cephalad as the external carotid arteries (Fig. 71).

By the end of the fourth day of incubation two more pairs of aortic arches have appeared posterior to the four formed in 55 to 60-hour chicks. From their first appearance the fifth aortic arches are very small and they soon disappear altogether. The first and second pairs of aortic arches have by this time suffered a marked diminution in size which is indicative of their final disappearance. In many embryos of this age the first arches, and in a few the second also, have disappeared altogether. This leaves only the third, fourth, and sixth pairs of aortic arches. These arches persist intact for some time, and parts of them remain permanently, being incorporated in he formation of the aortic arch and the main vessels arising from it, and in the roots of the pulmonary arteries.

In reptiles, birds, and mammals the main adult vessels which connect the heart with the dorsal aorta are derived from the fourth pair of aortic arches of the embryo. The paired condition of these arches persists as the adult condition in reptiles, but in birds and mammals one of the arches degenerates before the end of embryonic life. In birds the left arch degenerates leaving the right one as the adult aortic arch; in mammals the right arch degenerates leaving the left as the aortic arch of the adult.

The dorsal aortæ, at first paired, later become fused to form a median vessel. The fusion begins at about the level of the sinus venosus and progresses cephalad and caudad (Fig. 56). Fusion extends cephalad but a short distance, never involving the region of the aortic arches. Caudally the aortæ eventually become fused throughout their entire length.

Early in development the aorta gives rise to a segmentally arranged series of small vessels which extend into the dorsal body wall. At the level of the anterior appendage buds a pair of the segmental arteries become enlarged and extend into the wing buds as the subclavian arteries. Coincident with the

development of the allantois, segmental vessels opposite the allantoic stalk become enlarged and extend into it as the allantoic arteries. The external iliac arteries to the posterior appendage buds arise as branches of the allantoic arteries close to their origin from the aorta (Fig. 71).

In the adult, three vessels arising from the dorsal aorta supply the abdominal viscera. They are the cœliac, superior mesenteric, and the inferior mesenteric arteries. In four-day chicks these vessels are usually represented only by the omphalomesenteric arteries. The omphalomesenteric arteries arise as paired vessels (Fig. 56), but in the closure of the ventral body wall of the embryo they are brought together and fused to form a single vessel which runs in the mesentery from the aorta to the yolk-stalk (Figs. 74, *D* and 76). With the atrophy of the yolk-sac the proximal part of the omphalomesenteric artery persists as the superior mesenteric of the adult. The cœliac and the inferior mesenteric arteries arise from the aorta independently, usually at a somewhat later stage. Occasionally, however, the cœliac can be identified in four-day embryos (Fig. 71).

The cardinal veins are the principal afferent systemic vessels of the early embryo. They appear toward the end of the second day as paired vessels extending anteriorly and posteriorly on either side of the mid-line. At the level of the heart the anterior and posterior cardinal veins of the same side of the body become confluent in the ducts of Cuvier and turn ventrad to enter the sinus venosus (Figs. 42 and 59). Chicks of four days show little change in the relationships of the cardinal veins (Fig. 71). Later in development the proximal ends of the anterior cardinals become connected by the formation of a new transverse vessel and empty together into the right atrium of the heart. Their distal portions remain in the adult as the principal afferent vessels (jugular veins) of the cephalic region.

The posterior cardinals lie at first in the angle between the somites and the lateral mesoderm (Fig. 57, *D*, *E*). When the mesonephroi develop from the intermediate mesoderm, the posterior cardinal veins lie just dorsal to them throughout their length (Figs. 74, *D* and *E*, 75, *E-H*, 84, *C*). Situated ventrally in the mesonephroi are small irregular vessels roughly paralleling the posterior cardinals and anastomosing freely with them. These are the sub-cardinal veins (Fig. 71). They are

a relic of the renal portal circulation of more primitive ancestral forms, and are of importance in the embryos of birds and mammals chiefly because of the way they are involved in the formation of the posterior vena cava. In young embryos the posterior cardinals are the main afferent vessels of the posterior part of the body. Later in development they are replaced in this function by the posterior vena cava. In four-day chicks only the upper part of the posterior vena cava is indicated. It appears as a slender vessel extending from the liver caudally to anastomose with the right sub-cardinal vein. The formation of the vena cava, in part as a new vessel, and in part by appropriation of already existing vessels, and the changes by which the posterior cardinals become reduced and broken up to form small vessels with new associations, belong to stages of development beyond the scope of this book.

The Heart.—The heart in adult vertebrates is a ventral unpaired structure. Its origin in the chick from paired primordia is correlated with the way the young embryo lies spread out on the yolk surface. When the ventral body wall is completed by the folding together of layers which formerly extended to right and left over the yolk, the paired primordia of the heart are brought together in the mid-line. Their fusion establishes the heart as an unpaired structure lying in the characteristic ventral position (see Chap. IX and Figs. 44 and 45).

After the fusion of its paired primordia the heart is a nearly straight, double-walled tube (Figs. 37, and 79, *A*). The primordial endocardium of the heart has the same structure and arises in the same manner as the endothelial walls of the primitive embryonic blood vessels with which it is directly continuous. The epi-myocardial layer of the heart is an outer investment which surrounds and reinforces the endocardial wall. As development progresses the epi-myocardium becomes greatly thickened and is finally differentiated into two layers, a heavy muscular layer, the myocardium, and a thin non-muscular covering layer, the epicardium.

In the apposition of the paired primordia of the heart to each other, the splanchnic mesoderm from either side of the body comes together dorsal and ventral to the heart. The double-layered supporting membranes thus formed are known as the

dorsal mesocardium and the ventral mesocardium, respectively (Fig. 44). The ventral mesocardium disappears shortly after its formation, leaving the heart suspended in the body cavity by the dorsal mesocardium (Fig. 44, *E, D*). Somewhat later the dorsal mesocardium also disappears except at the caudal end of the heart. Thus the heart comes to lie in the pericardial cavity unattached except at its two ends. The cephalic end of the heart remains fixed with reference to the body of the embryo where the ventral aorta lies embedded ventral to the floor of the pharynx, and the caudal end of the heart is fixed by the persistent portion of the dorsal mesocardium and the omphalomesenteric veins.

The straight tubular condition of the heart persists but a short time. The unattached ventricular region becomes dilated and is bent out of the mid-line toward the embryo's right while the fixed bulbo-conus arteriosus and the sinus venosus are held in their original median position (Fig. 79, *A–E*). This bending of the heart to form a U-shaped tube begins to be apparent in embryos of 30 hours and becomes rapidly more conspicuous, until by forty hours the ventricular region of the heart lies well to the right of the embryo's body (Fig. 78).

The bending of the heart to the side involves a considerable factor of "mechanical expediency." The initiation of the bending process depends on the fact that the heart is becoming elongated more rapidly than is the chamber in which it lies fixed by its two ends. The fact that the bending takes place to the side rather than dorsally or ventrally may be attributed to the impediment offered to its dorsal bending by the body of the embryo, and to its ventral bending by the yolk.

The lateral bending of the heart attains its greatest extent at about 40 hours of incubation. At this stage torsion of the body of the embryo changes the mechanical limitations in the heart region. As the embryo comes to lie on its left side the heart is no longer pressed against the yolk (Cf. Figs. 78, *A* to *F*). As a result the bent ventricular region begins to swing somewhat ventrad and lies less closely against the body of the embryo (Figs. 78 and 80, *E* and *F*).

At about this stage of development the closed part of the U-shaped bend becomes twisted on itself to form a loop (Figs.

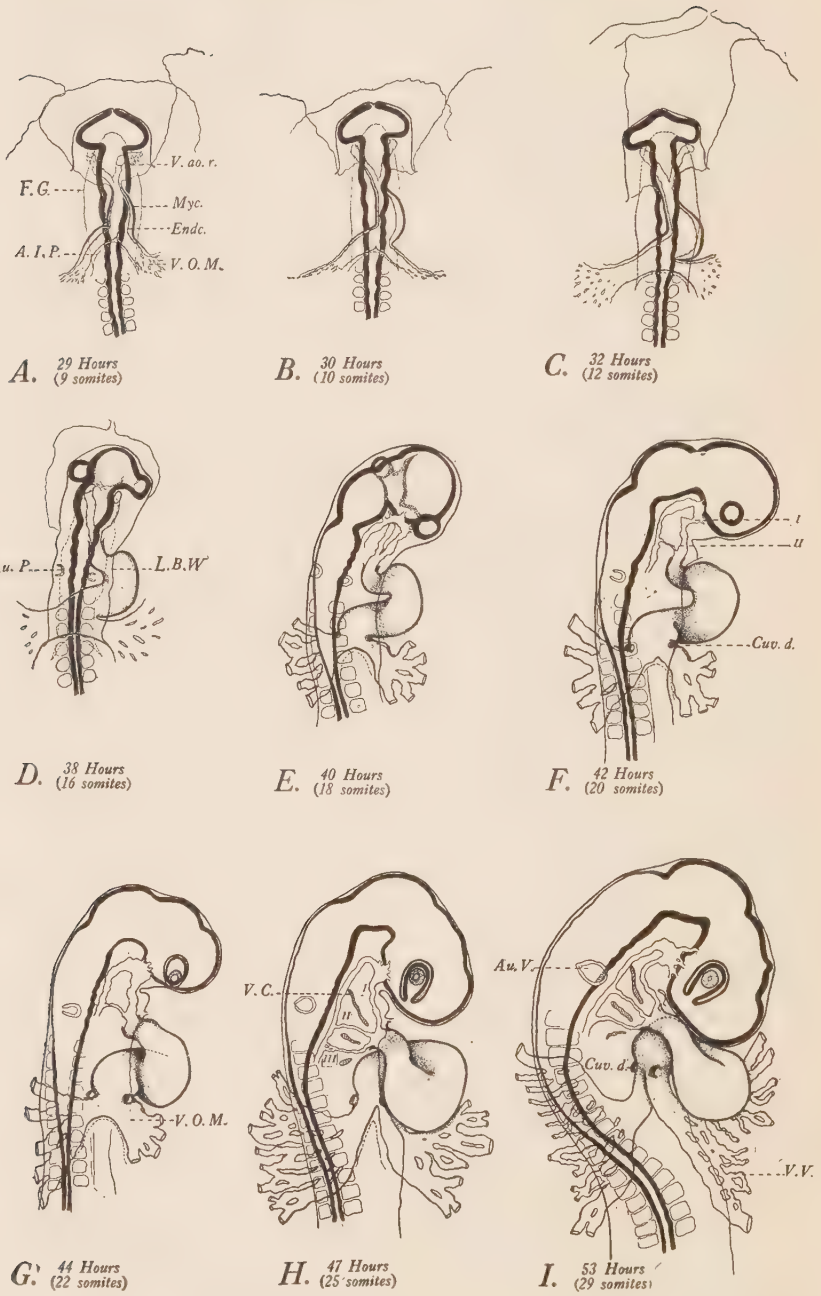
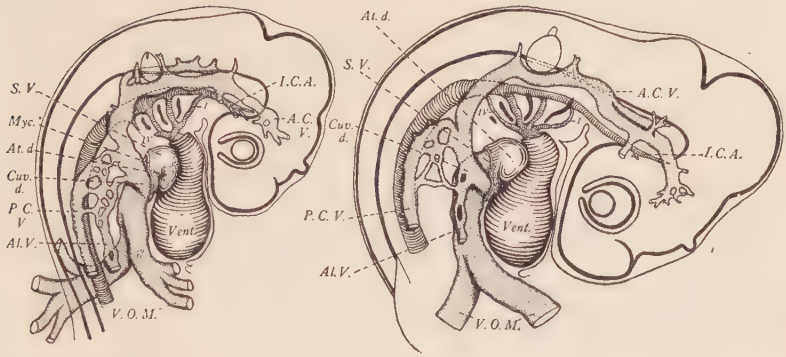
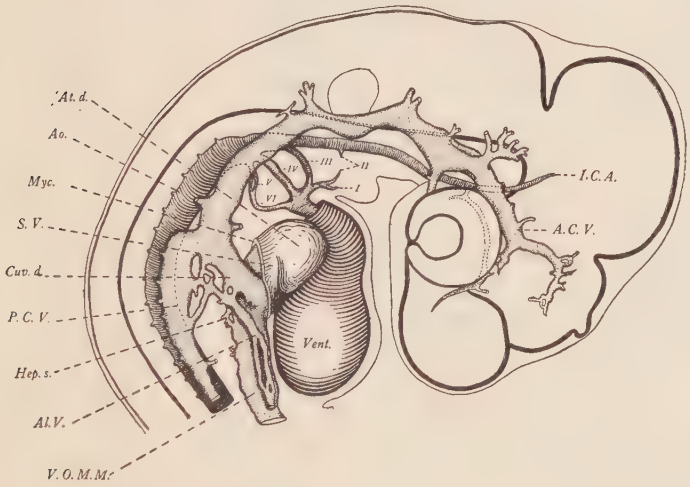

V. ao. r.
F.G.
Myc.
Endc.
A. I. P.
V. O. M.
A. 29 Hours (9 somites)
B. 30 Hours (10 somites)
C. 32 Hours (12 somites)
Au. P.
L.B.W.
D. 38 Hours (16 somites)
E. 40 Hours (18 somites)
t
u
Cuv. d.
F. 42 Hours (20 somites)
V. O. M.
G. 44 Hours (22 somites)
V. C.
H. 47 Hours (25 somites)
Au. V.
Cuv. d.
V. V.
I. 53 Hours (29 somites)

FIG. 78.



J. 65 Hours (33 somites) *K.* 76 Hours (38 somites)



L. 100 Hours (45 somites)

FIG. 78. *A* to *L.*—Camera outlines (× 15) of cephalic and cardiac regions of chick embryos of various ages showing the relations of the heart and great vessels. This figure is arranged and lettered to correspond with the detailed drawings of the heart in Figs. 79, 80 and 81:

Key to abbreviations: *I* to *VI*, aortic arches I to VI, respectively; *A.C.V.*, anterior cardinal vein; *A.I.P.*, anterior intestinal portal; *Al.V.*, allantoic vein; *Ao.*, aorta; *At.*, atrium (*d.*, right), (*s.*, left); *Au.P.*, auditory pit; *Au.V.*, auditory vesicle; *Bul.*, bulbus cordis; *Cuv.d.*, duct of Cuvier; *Endc.*, endocardium; *F.G.*, foregut; *Hep.s.*, stubs of some of the larger hepatic sinusoids; *I.C.A.*, internal carotid artery; *L.B.W.*, lateral body wall; *Myc.*, epi-myocardium; *Myc*.*, cut edge of epi-myocardium; *P.C.V.*, posterior cardinal vein; *Sin.-at.*, sino-arterial region of heart before its definite division; *S.V.*, sinus venosus; *Vent.*, ventricle; *V.ao.r.*, ventral aortic roots; *V.C.*, visceral cleft; *V.O.M.*, omphalomesenteric veins; *V.O.M.M.*, fused omphalomesenteric veins; *V.V.*, vitelline veins.

78, 79, 80, and 81, *F–I*). In the formation of the loop the atrial region is forced slightly to the left (*i.e.*, toward the yolk) and the conus, or as it is now preferably called the truncus arteriosus,

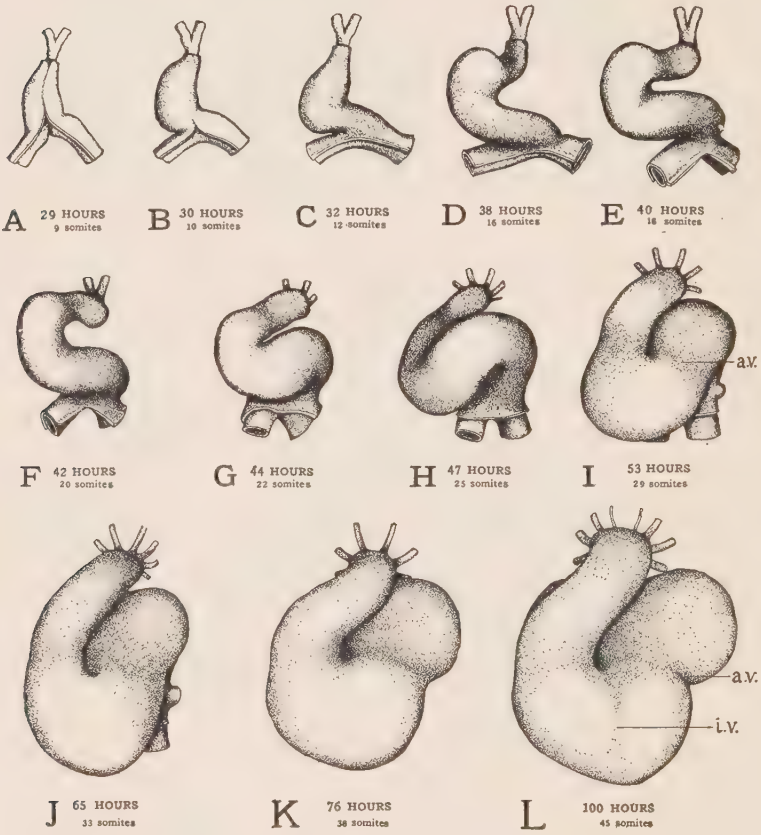

A 29 HOURS 9 somites
B 30 HOURS 10 somites
C 32 HOURS 12 somites
D 38 HOURS 16 somites
E 40 HOURS 18 somites
F 42 HOURS 20 somites
G 44 HOURS 22 somites
H 47 HOURS 25 somites
I 53 HOURS 29 somites
a.v.
J 65 HOURS 33 somites
K 76 HOURS 38 somites
L 100 HOURS 45 somites
a.v.
i.v.

FIG. 79.—Ventral views of the heart at various stages to show its changse of shape and its regional differentiation. All the drawings were made from dissections with the aid of camera lucida outlines. The outer of the two layers shown is the epi-myocardium; the inner, the endocardium. In the stages represented in Figs. *E-H* torsion of the embryo's body is going on at the level of the heart. Since torsion involves the more cephalic regions first and progresses caudad the transverse axis of the body of the embryo is at different inclinations to the yolk at the cephalic end and at the caudal end of the heart. In drawing these figures their orientation was taken from the body at the level of the conus region of the heart, the sinus region therefore appears inclined. Abbreviations: a.v., constriction between atrium and ventricle; i.v., interventricular groove.

is thrown across the atrial region by being bent to the right (*i.e.*, away from the yolk) and then dorso-caudad. The ventricular region constituting the closed end of the loop swings dorsalwards and toward the tail, possibly being crowded in this

direction by the increasing flexion of the cephalic part of the embryo (Fig. 78, *G* to *J*). Thus the original cephalo-caudal relations of the atrial and ventricular regions are reversed and the atrial region which was at first caudal to the ventricle now lies cephalic to it as in the adult heart.

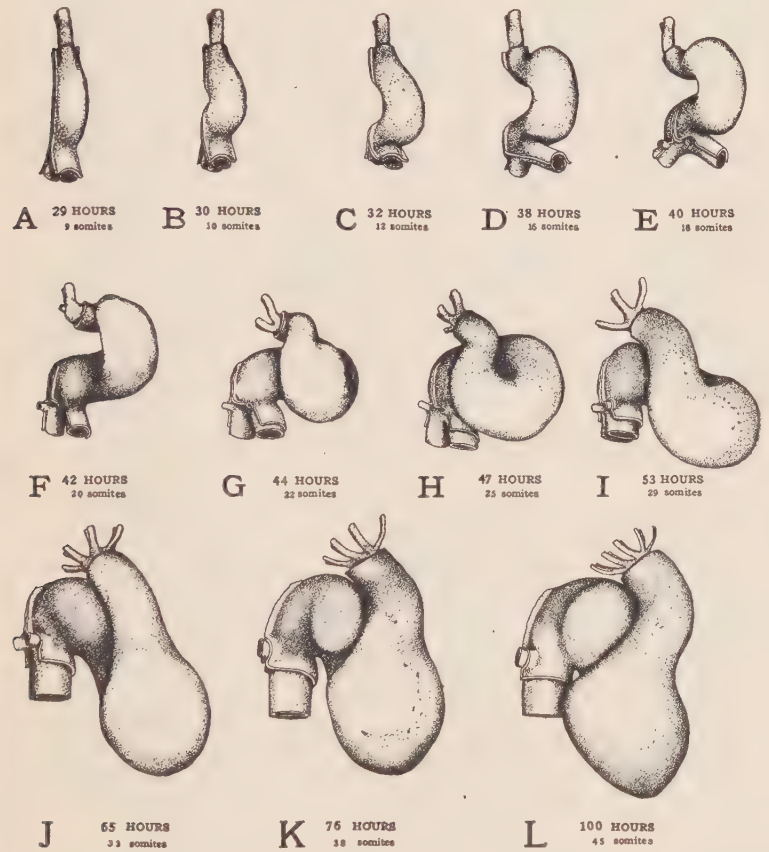

A 29 HOURS 9 somites
B 30 HOURS 10 somites
C 32 HOURS 12 somites
D 38 HOURS 16 somites
E 40 HOURS 18 somites
F 42 HOURS 20 somites
G 44 HOURS 22 somites
H 47 HOURS 25 somites
I 53 HOURS 29 somites
J 65 HOURS 33 somites
K 76 HOURS 38 somites
L 100 HOURS 45 somites

FIG. 80.—Dextral views of the same series of hearts shown in ventral view in Fig. 79 and in dorsal view in Fig. 81. The heart drawings in Figs. 79, 80 and 81 should be compared with actual specimens or with drawings of entire embryos of corresponding age for the relation of the heart to the body of the embryo. (See Fig. 78.)

The atrial region and the ventricular region which formerly were continuous without any line of demarcation, are by this time beginning to be marked off from each other by a constriction (Fig. 79, *I*, a.v.). As both the atrium and the ventricle become enlarged, this constriction is accentuated (Fig. 79, *L*, a.v.). The constricted region is now termed the atrio-ventricular canal.

During the fourth day the truncus arteriosus becomes closely applied to the ventral surface of the atrium. As the atrium grows it tends to expand on either side of the depression

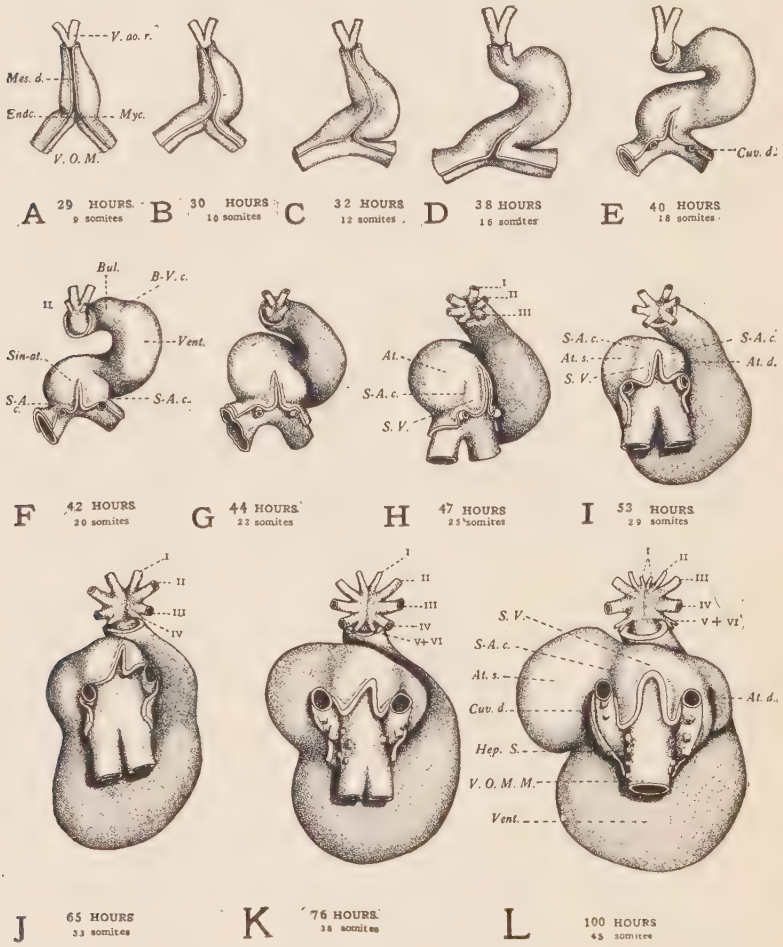


FIG. 81.—Dorsal views of same series of hearts shown in Fig. 79 in ventral view, and in Fig. 80 in lateral view.

ABBREVIATIONS: *I-VI*, aortic arches I to VI; *At.*, atrium (*d.* right), (*s.* left); *Bul.*, bulbus cordis; *B-V.c.*, bulboventricular constriction; *Cuv.d.*, duct of Cuvier; *Endc.*, endocardium; *Hep.s.*, stubs of some of the larger hepatic sinusoids; *Mes.d.*, dorsal mesocardium; *Myc.*, cut edge of epi-myocardium; *S-A.c.*, sino-atrial constriction; *Sin-at.*, sino-atrial region (before its definite division); *S.V.*, sinus venosus; *C.ao.r.*, ventral aortic roots; *Vent.*, ventricle; *V.O.M.*, omphalomesenteric veins; *V.O.M.M.*, fused omphalomesenteric veins.

made in it by the pressure of the truncus (Figs. 79, *J–L* and 81, *J–L*). These lateral expansions are the first indication of the division of the atrium into right and left chambers which are

later completely separated from each other. At the same time a slight longitudinal groove appears in the surface of the ventricle (Fig. 79, *L*, i.v.) which indicates the beginning of the separation of the ventricle into right and left chambers.

During the changes in the external shape of the heart which have been described, the whole heart has come to occupy a more caudal position with reference to other structures in the embryo. When the heart is first formed it lies at the level of the rhombencephalon. As development progresses it moves farther and farther caudad until at the end of the fourth day it lies at the level of the anterior appendage buds (Fig. 78).

The changes which take place in the heart wall can be seen best in sections. The endocardium in the heart of a four-day chick is still a single cell layer lining the lumen. The original epi-myocardium at this stage can be differentiated into an inner, myocardial portion and an outer, epicardial portion. The myocardium has become greatly thickened and the cells in it are elongated and beginning to show the histological characteristics of developing muscle cells. Their arrangement in bundles which project toward the lumen fore-shadows the formation of the muscle bands (trabeculæ carneæ) which ridge the inner wall of the adult heart. The cells of the epicardial portion of the epi-myocardium are becoming flattened to form the epithelial and connective tissue covering of the heart (epicardium). Lying between the endocardium and the myocardium in the region of the atrio-ventricular canal and of the opening of the ventricle into the truncus arteriosus, there are loosely aggregated masses of cells which are mesenchymal in their characteristics. These cells constitute what is called endocardial cushion tissue. This plastic connective tissue later takes part in the partitioning of the atrio-ventricular canal and in the formation of the connective tissue frame-work of the cardiac valves.

The definite establishment of the four-chambered condition characteristic of the adult heart belongs to later stages of development than those under consideration. By the end of the fourth day, however, the partitioning process is already begun. In reconstructions showing the interior of the heart the interatrial septum appears as a sickle-shaped partition cutting into the atrial lumen along the line where it is already narrowed by the pressure of the truncus arteriosus (Fig. 82).

On the interior of the ventricular wall, opposite the interventricular groove (Fig. 79, *L*), the trabeculæ of growing muscle are especially abundant and project well into the ventricular lumen (Fig. 82). Consolidation of these trabeculæ establishes a definite septum which grows from the apex of the ventricle toward the atrio-ventricular canal. Convergent growth of

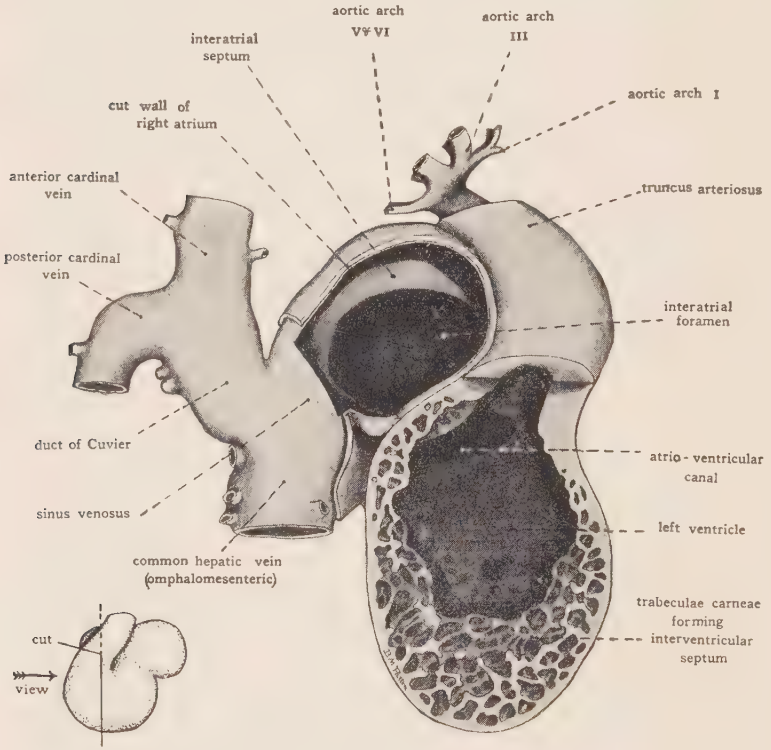


FIG. 82.—Reconstruction of the heart of a four-day chick. (*Patten and Zimmer.*) The model is drawn as viewed from the left when cut open along the lines indicated in the orienting sketch.

the interatrial and interventricular septa, and their ultimate fusion with a partition later established in the atrio-ventricular canal, finally accomplish the division of the heart into right and left sides. Concomitantly the truncus arteriosus is divided into pulmonary and aortic channels communicating respectively with the right and left ventricle. With the completion of these processes the heart is prepared to pump separate pulmonary and systemic blood streams.

VI. The Urinary System

The General Relationship of Pronephros, Mesonephros and Metanephros.—In the development of the urinary system of birds and mammals there are formed in succession three distinct excretory organs, pronephros, mesonephros, and metanephros. The pronephros is the most anterior of the three, and the first to be formed. It is wholly vestigial, appearing only as a slurred-over recapitulation of structural conditions which exist in the adults of the most primitive of the vertebrate stock. The mesonephros is homologous with the adult excretory organs of fishes and amphibia. It makes its appearance in the embryo somewhat later than the pronephros, and is formed caudal to it. The mesonephros is the principal organ of excretion during early embryonic life, but it also disappears in the adult except for parts of its duct system which become associated with the reproductive organs. The metanephros is the most caudally located of the excretory organs, and the last to appear. It becomes functional toward the end of embryonic life when the mesonephros is disappearing, and persists permanently as the functional kidney of the adult.

Figure 83 shows schematically some of the main steps in the embryological history of the nephric organs, which it will be helpful to have in mind before taking up in detail any of the phases of their formation in the chick. The pronephros, mesonephros and metanephros are all derived from the intermediate mesoderm, and are all composed of units which are tubular in nature. In the different nephroi these tubules vary in structural detail but their functional significance is in all cases much the same. They are concerned in collecting waste materials from the capillary plexuses which are developed in connection with them. In the accompanying diagrams conventionalized tubules have been drawn to represent the three nephric organs. No pretense is made of representing either the exact shape or the actual number of the tubules.

In the first stage represented (Fig. 83, *A*) only the pronephros has been established. It consists of a group of tubules emptying into a common duct, called the pronephric duct. The pronephric ducts of either side are formed first at the level of the pronephric tubules and then extend caudad, eventually reaching and opening into the cloaca (See arrows in Fig. 83, *A*).

As the pronephric ducts are extended caudal to the level at which pronephric tubules are formed they come in close prox-

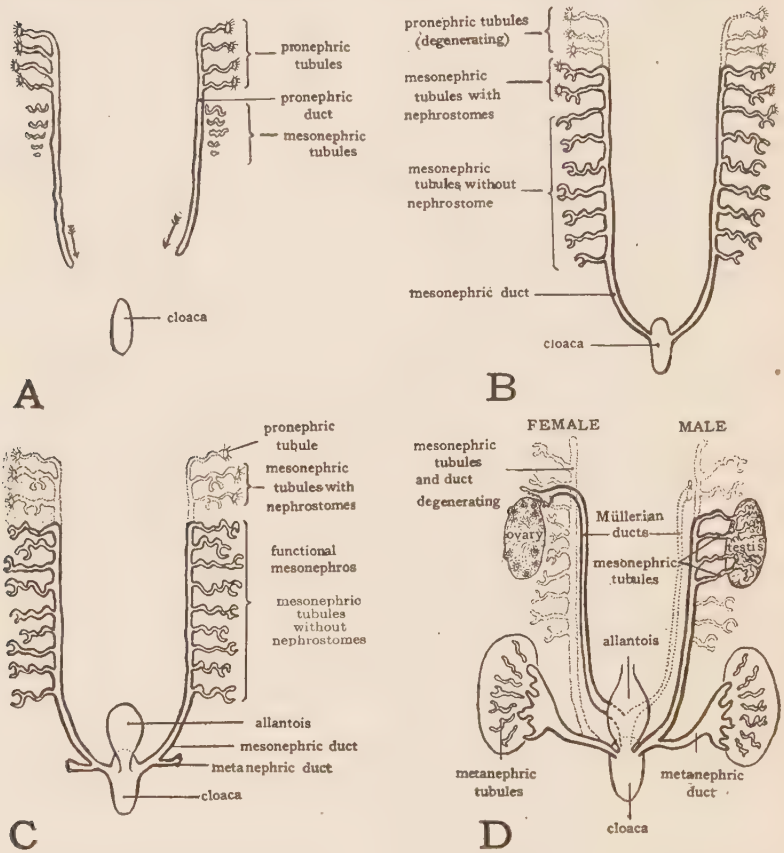


FIG. 83.—Schematic diagrams to show the relations of pronephros, mesonephros, and metanephros at various stages of development. For the sake of simplicity the tubules have been drawn as if they had been pulled out to the side of the ducts. Their actual positional relations within the body are shown in figure 85.

The plan as drawn in *C* represents approximately the conditions attained by the chick at the end of the fourth day of incubation. In *D* are depicted the conditions after sexual differentiation has taken place. The Müllerian ducts (shown in *D*) arise, during the chick's fifth day of incubation, in close association with the mesonephric ducts. The Müllerian ducts are the primordial tubes from which the oviducts, uterus and vagina of the female are formed. Note that while both mesonephric and Müllerian ducts appear in all young embryos the Müllerian ducts become vestigial in the males and the mesonephric ducts become vestigial in the females.

imity to the developing mesonephric tubules. In their growth the mesonephric tubules extend toward the pronephric ducts and soon open into them (Fig. 83, *B*). Meanwhile the pro-

nephric tubules begin to degenerate. Thus the ducts which originally arose in connection with the pronephros are appropriated by the developing mesonephros. After the degeneration of the pronephric tubules these same ducts are called the mesonephric ducts because of their new associations (Fig. 83, *C*).

At a considerably later stage outgrowths develop from the mesonephric ducts near their cloacal ends (Fig. 83, *C*). These outgrowths form the ducts of the metanephroi. They grow cephalo-laterad and eventually connect with the third group of tubules developed from the intermediate mesoderm, the metanephric tubules (Fig. 83, *D*). With the establishment of the metanephroi or permanent kidneys the mesonephroi begin to degenerate. The only parts of the mesonephric system to persist, except in vestigial form, are some of the ducts and tubules which, in the male, are appropriated by the testis as a duct system.

The Pronephric Tubules of the Chick.—The pronephros in the chick is represented by tubules which first appear at about 36 hours of incubation. The pronephric tubules arise from the intermediate mesoderm, or nephrotome, lateral to the somites. They are paired, segmentally arranged structures, a tubule appearing on either side opposite each somite from the fifth to the sixteenth. Transverse sections passing through the 10th to 14th somites of an embryo of about 38 hours show the pronephric tubules favorably. Each tubule arises as a solid bud of cells organized from the intermediate mesoderm near its junction with the lateral mesoderm (Fig. 84, *A*). At first the free ends of the buds grow dorsad, passing close to the posterior cardinal veins. Later the end of each tubule is bent caudad coming in contact with the tubule lying posterior to it. In this manner the distal ends of the tubules give rise to a continuous cord of cells, the primordium of the pronephric duct. The pair of cell cords thus formed continue to extend caudad beyond the pronephric tubules and soon become hollowed out to form open ducts. When they eventually reach the level of the cloaca they turn ventrad and open into it.

The significance of the rudimentary structures in the chick which represent pronephric tubules, can most readily be understood by comparing them with fully developed and functional pronephric tubules. Figure 84, *B*, shows the scheme of

organization of a functional pronephric tubule. The ciliated nephrostome draws in fluid from the cœlom. As the fluid passes the capillaries of the glomus, waste materials from the blood are transferred to it. The nephric duct serves to collect and discharge the fluid passing through the tubules. In the

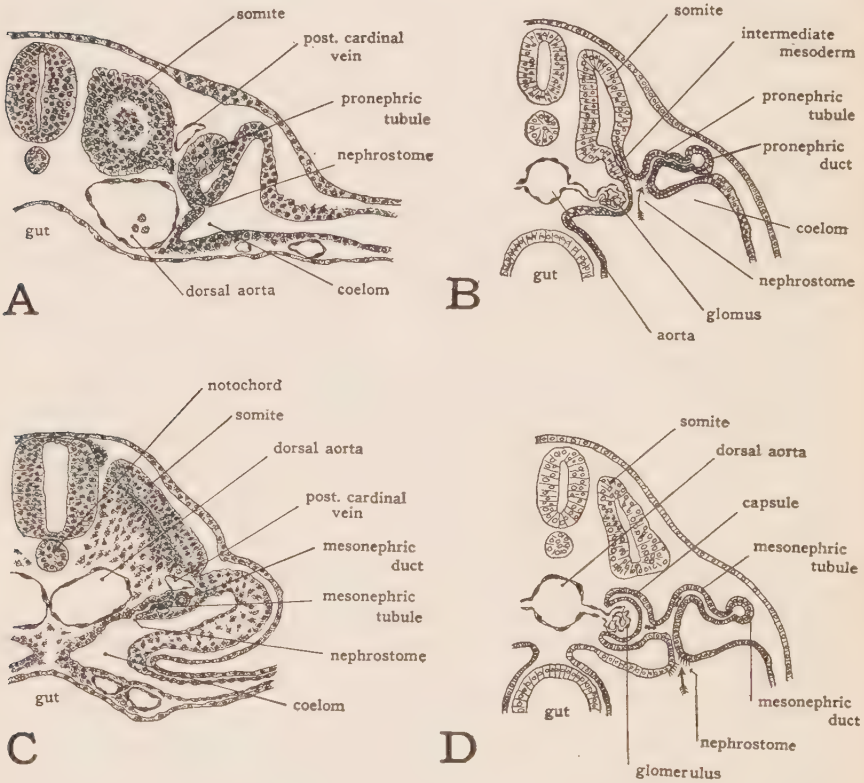


FIG. 84.—Drawings to show nephric tubules. *A*, drawing from transverse section through twelfth somite of 16-somite chick to show pronephric tubule. (*After Lillie.*) *B*, schematic diagram of functional pronephric tubule. (*After Wiedersheim.*) *C*, drawing from transverse section through seventeenth somite of 30-somite chick to show primitive mesonephric tubule; *D*, schematic diagram of functional mesonephric tubule of primitive type. (*After Wiedersheim.*) For a later stage of the mesonephric tubules of the chick see Fig. 85.

pronephric tubules of the chick there are vestiges of a nephrostome opening into the cœlom (Fig. 84, *A*) but the tubules never become completely patent, and never acquire the vascular connections characteristic of the functional pronephros in primitive vertebrates. Shortly after their initial appearance the pronephric tubules begin to undergo regressive changes

and by the end of the fourth day of incubation a few isolated epithelial vesicles are all that remain to chronicle the transitory appearance of the pronephros.

The Mesonephric Tubules.—The mesonephric tubules develop from the intermediate mesoderm caudal to the pronephros. The early steps in their formation are well shown in transverse sections of chicks of 29 to 30 somites (about 55 hours). In the posterior region conditions are less advanced than they are more anteriorly. Consequently by studying the posterior sections of a transverse series first and then progressing cephalad a graded series of developmental stages may be obtained.

The mesonephric tubules appear first as cell clusters formed in the intermediate mesoderm ventro-mesial to the cord of cells which is the primordium of the pronephric duct. The cells of the developing tubules acquire a more or less radial arrangement, and at the same time become more distinctly isolated from the surrounding mesoderm cells. By 55 hours of incubation the primordial cell cord representing the pronephric duct has become hollowed out to establish a definite lumen. The most anterior of the mesonephric tubules also have acquired a lumen. Meanwhile the growth of the tubules has brought them in close association with the duct and in some of the more differentiated tubules indications can be made out of their opening into the duct. The more posterior mesonephric tubules do not become associated with the duct until somewhat later, but remain as a series of isolated vesicles.

Figure 84, *D*, shows the scheme of organization of a functional mesonephric tubule of primitive type. As is the case with the pronephric tubule, its ciliated nephrostome draws in fluid from the cœlom. The mesonephric tubule differs from the pronephric chiefly in its relation to the blood vessels associated with it. It develops a cup-like outgrowth into which a knot of capillaries is pushed. The cup-shaped outgrowth from the tubule is called the capsule (of Bowman) and the tuft of capillaries, a glomerulus. Waste-laden fluid is extracted from the capillaries of the glomerulus, mingles with the fluid coming in by way of the nephrostome, and is eventually discharged into the nephric duct. In mesonephric tubules of a more highly differentiated type the nephrostome becomes closed and all

the fluid passing through the tubule is drawn from the glomerulus and other capillaries adjacent to the tubule.

In the chick a few of the more anterior mesonephric tubules are of the primitive type and show vestiges of a nephrostome opening into the cœlom (Fig. 84, *C*). These anterior mesonephric tubules, however, persist for but a short time, do not attain the characteristic relation to a glomerulus, and never become functional. In chicks of four day's incubation the mesonephric tubules have not attained their full development but it is possible to make out most of their fundamental

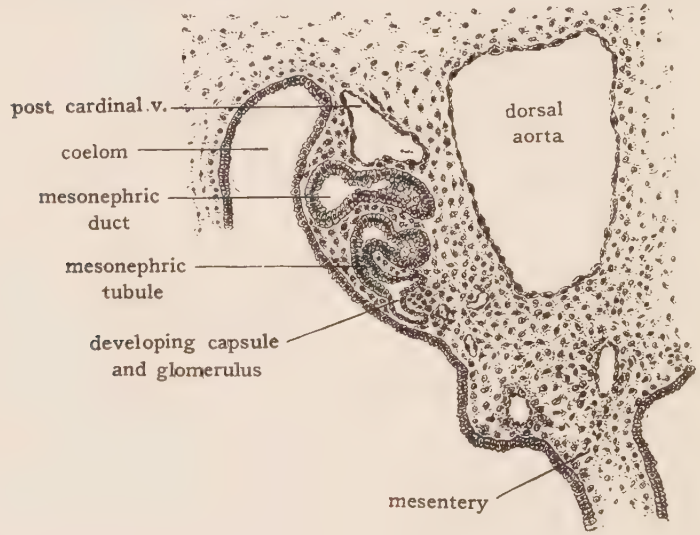


FIG. 85.—Drawing from transverse section of four-day chick to show mesonephric tubule and duct. For the location of the area drawn consult Fig. 75, *F*.

parts (Fig. 85). The tubules lying ventro-laterally in the cephalic part of the mesonephros have been longest established and are somewhat more advanced in development than those lying in other parts of the mesonephros. Nearly all of the tubules, however, have become elongated and somewhat coiled. At one end they open into the mesonephric duct or a diverticulum of the duct which acts as a collecting tubule. At their other end a cluster of closely packed cells indicates the place at which the capsule and glomerulus will appear. Once established the glomeruli develop very rapidly. Circulation usually commences in them by the fifth day. From this time until

about the eleventh day of incubation the functional activity of the mesonephros is at its height. After the eleventh day the developing metanephros begins to become active and the mesonephros degenerates. The establishment of the metanephros and the development of the genital organs occur in stages which are too advanced to come within the scope of this book.

VII. The Cœlom and Mesenteries

In adult birds and mammals the body cavity consists of three regions, pericardial, pleural, and peritoneal. The pleural cavities are paired, each of the pleural chambers being a laterally situated sac containing one of the lungs. The pericardial chamber containing the heart, and the peritoneal chamber containing the viscera, other than the lungs and heart, are unpaired. These regions of the adult body cavity are formed by the partitioning off of the primary body cavity or cœlom of the embryo.

In the chick the cœlom arises by a splitting of the lateral mesoderm of either side of the body (Fig. 86, *A*, *B*). It is, therefore, primarily a paired cavity. Unlike the cœlom of some of the more primitive vertebrates, the cœlom of the chick never shows any indications of segmental pouches corresponding in arrangement with the somites. The right and left cœlomic chambers extend antero-posteriorly without interruption through the entire lateral plates of mesoderm. This difference in the formation of the cœlom does not imply any lack of homology between the cœlom of the chick and that of more primitive forms. The process of cœlom formation in the chick may be considered as being accelerated with a resultant slurring over of the early phases. Or, to state the matter in another way, the cœlom of birds and mammals first appears in a condition which is comparable with the cœlom of more primitive forms at that period of differentiation when the segmentally arranged cœlomic pouches have broken through into each other and their cavities have become confluent.

The cœlomic chambers are not limited to the region in which the body of the embryo is developing. They extend on either side into the mesoderm, which in common with the other germ layers, spreads out over the yolk surface. A large part of the primitive cœlomic chambers thus comes to be extra-embryonic

in its associations. (See Chapter XI and Figures 49 and 52.) The portion of the cœlom which gives rise to the embryonic body cavities is first marked off by the series of folds which

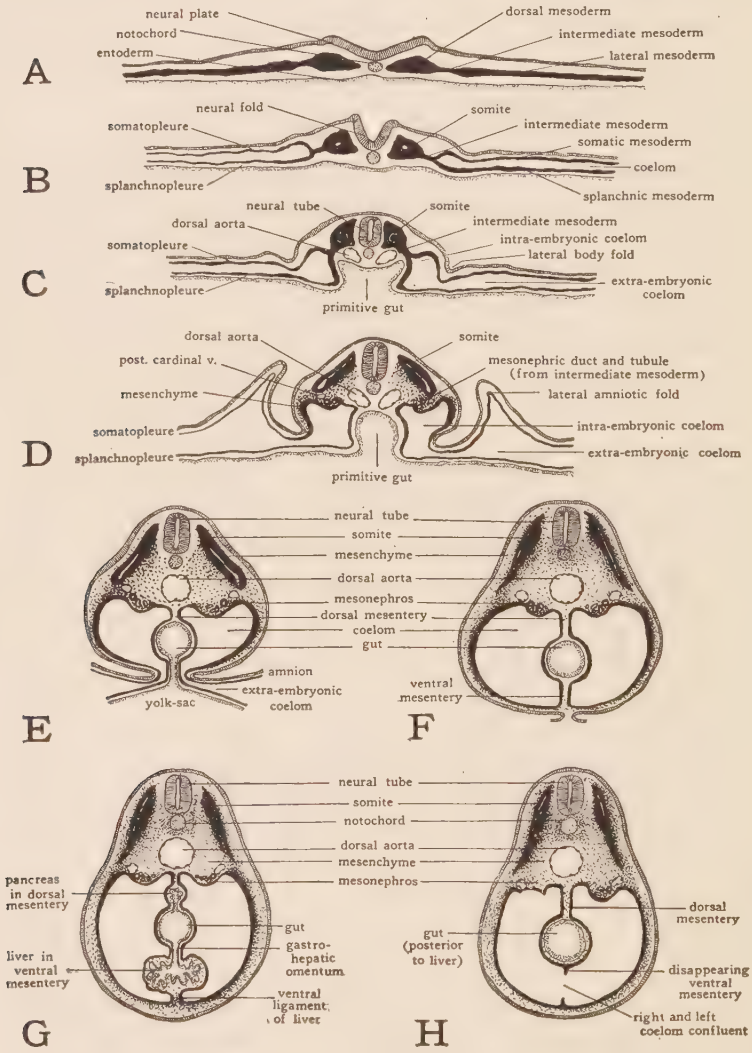


FIG. 86.—Schematic diagrams of cross sections at various stages to show the establishment of the cœlom and mesenteries. For explanation see text.

separate the body of the embryo from the yolk (Fig. 86, *C*, *D*). As the closure of the ventral body wall progresses (Fig. 86, *E*, *F*) the embryonic cœlom becomes completely separated from the extra-embryonic. The delayed closure of the ventral body

wall in the yolk-stalk region, results in the embryonic and extra-embryonic cœlom retaining their open communication at this point for a long time after they have been completely separated elsewhere.

The same folding process which establishes the ventral body wall completes the gut ventrally (Fig. 86, *C* to *F*). Meanwhile the right and left cœlomic chambers are expanded mesiad. As a result the newly closed gut comes to lie suspended between the two layers of splanchnic mesoderm which constitute the mesial walls of the right and left cœlomic chambers. The double layer of splanchnic mesoderm which thus becomes apposed to the gut and supports it in the body cavity is known as the mesentery. The part of the mesentery dorsal to the gut, suspending it from the dorsal body wall, is the primary dorsal mesentery, and the part ventral to the gut, attaching it to the ventral body wall, is the primary ventral mesentery.

When the dorsal and ventral mesenteries are first established they constitute a complete membranous partition dividing the body cavity into right and left halves. The primary dorsal mesentery persists in large part but the ventral mesentery early disappears bringing the right and left cœlomic chambers into confluence ventral to the gut and establishing the unpaired condition of the body cavity characteristic of the adult.

In considering the early development of the heart (Chapter IX) the formation of the dorsal and ventral mesocardia was taken up. In their relation to the other mesenteries of the body, the mesocardia may be regarded as special regions of the primary ventral mesentery. In the most cephalic part of the body cavity, the gut lies embedded in the dorsal body wall instead of being suspended by the primary dorsal mesentery as it is farther caudally (Cf. Fig. 44, *E* and Fig. 86, *F*). A ventral mesentery is, however, developed in the same manner anteriorly as it is posteriorly, and when the heart is formed it is suspended in the most anterior part of this ventral mesentery. The dorsal and ventral mesocardia may, therefore, be thought of as the parts of the primary ventral mesentery lying dorsal to the heart, and ventral to the heart, respectively (Fig. 44, *D*).

When the ventral mesocardium, and a little later the dorsal mesocardium, breaks through, the primary right and left cœlomic chambers become confluent to form the pericardial

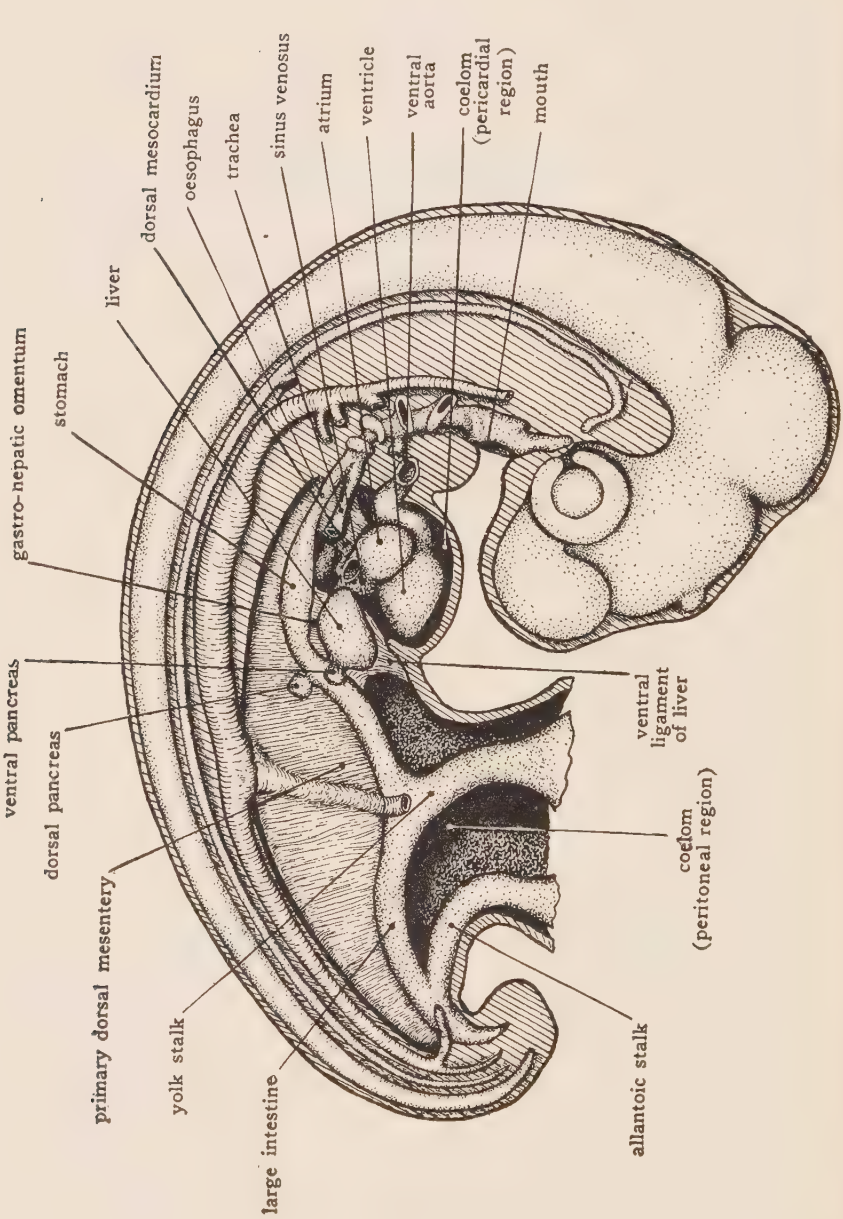


FIG. 87.—Schematic lateral view of dissection of four-day chick to show the body cavity and the more important mesenteries.

region of the body cavity (Figs. 42 and 87). Later in development the ventral mesentery farther caudally disappears, so that caudally as well as cephalically an unpaired condition of the cœlom is brought about (Fig. 86, *H*).

In the liver region a portion of the ventral mesentery remains intact. The liver arises as an outgrowth from the gut and in its development extends into the ventral mesentery (Fig. 86, *G*). That portion of the ventral mesentery which is dorsal to the liver persists as the gastro-hepatic omentum, and that portion of the mesentery which is ventral to the liver persists as the falciform ligament or ventral ligament of the liver (Fig. 87).

The entire primary dorsal mesentery persists and forms the supporting membranes of the digestive tube. In the adult its different regions are named according to the parts of the digestive tube with which they are associated, as for example, mesogaster, that part of the primary dorsal mesentery which suspends the stomach, mesocolon, that part of the primary dorsal mesentery supporting the colon, etc.

The separation of the body cavity into pericardial, pleural, and peritoneal chambers is accomplished by the formation of septa growing in from the body wall. Consideration of the details of their formation would lead us into stages of development beyond the scope of this book. Those interested in following this or other phases of the later embryology of the chick will find in the appendix references to more exhaustive books, and to some of the more recent original papers on its development.

APPENDIX

BIBLIOGRAPHY

While this does not purport to be a complete bibliography on the embryology of the chick I have attempted to make it comprehensive. At least one reference has been included on every phase of chick development concerning which published information could be located. At the same time an effort has been made to keep the list within reasonable bounds by limiting it largely to recent articles in accessible publications. With the exception of a few classic contributions, papers of merely historical interest have not been included. Those interested in this aspect of the subject can find, under each of the main headings, articles having extensive bibliographies covering the old as well as the current literature in their special fields. It is hoped that the instructor will find the references a convenience in selecting collateral reading, and especially that the student who becomes spontaneously interested in knowing more about some special phase of the subject can find references which will start him toward acquiring the information he desires.

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INDEX

To facilitate the use of this book in connection with others in which the terminology may differ somewhat, many synonyms which were not used in the text have been put into the index and cross-referenced to the alternative terms used in this book. For example, Wolffian body, a term not used in this text, is frequently applied to the mesonephros. It appears in the index thus: Wolffian body (= mesonephros, q.v.).

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